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ON EXPERIMENTAL TREMOR

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MEĐUNARODNI SIMPOZIJUM

O EKSPERIMENTALNOM TREMORU

1 — 3. APRIL 1971. GODINE

Urednik
PAVEL STERN,
redovni član Akademije nauka i umjetnosti
Bosne i Hercegovine



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Editor
PAVEL ŠTERN,
member of Academy of sciences and arts
of Bosnia and Herzegovina



SARAJEVO
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EDITOR'S PREFACE

After more than two years of preparing, the long-planned idea of organizing a Symposium of experimental tremor has come to reality. In the beginning we thought only of meeting for experimental tremor and rigor, but later on we found out that there is a great interest of other scientists who are engaged in clinical medicine, so at last we organized a conference of tremor from different aspects. The attention was made to the aethyology of human tremor, as it can be seen from the lectures and from the discussion, too. According to this, the theme of Conference was widened, and is not quite suitable to the title of the Symposium.

Using this chance, I want to thank again to Chairmanship of Academy of Sciences and Art of BiH and to Federal Council for scientific work in Belgrade for the financial help. In the same way I thank to the medical factories: »Bosnalijek« (Sarajevo), »Pliva« (Zagreb), »Krka« (Novo Mesto), »Lek« (Ljubljana), Boehringer und Sohn (Ingelheim), Knoll A. G. (Ludwigschafen), »Lepetit« (Milano), Merck E. (Darmstadt), »Troponwerke« (Köln), and to Genaral Hospital Tuzla for financial help.

At last I wish to thank to all my collaborators for their help before and during the Symposium.

P. Štern

P. ŠTERN
POZDRAVNA RIJEČ

Sastali smo se da prodiskutiramo o današnjoj situaciji bolesti uzrokovane metaboličkim promjenama u striatumu i da utvrdimo kako se te bolesti mogu najbolje ispitivati u eksperimentu na ljudima i životinjama. Simpoziji ovoga karaktera u zadnjih deset godina nisu rijetki, što u prvom redu govori za aktuelnost teme i drugo potvrđuje da se u kratkom vremenu nagomilavaju mnoge činjenice koje treba prema tome i kritički razmotriti i konfrontirati sa starijim spoznajama. Znamo da su biokemijske promjene u striatumu, koje dovode do promjena u smislu Parkinsona ili Wilsonove bolesti postale pristupačne i jasnije tek u zadnjih 12 godina. Od onda do danas kolosalno mnogo je publicirano na tom polju i u smislu fundamentalnog istraživanja na životinjama ili klinički na ljudima sa nemalim uspjesima u terapiji. Ovako veliki napredak u poznavanju biokemijskih promjena kao kod bolesti striatuma nije do sada postignut ni kod jedne druge grupe neuroloških ili psihijatrijskih oboljenja. To nužno zahtijeva daljnje istraživanje i dobre modele na životinjama kako bi se omogućio nastavak ovakvog tempa i intenziteta dobivanjem novih informacija. Zahvaljujući jednom dobrom modelu prof. Zec i ja smo mogli pred jedno 8 godina pokazati na pr. da alfa-methyl-dopa daje dobar terapijski efekt ne samo na životinjama nego i humanoj patologiji kod slučajeva Wilsonove bolesti ili choree minor. To je potvrđeno i od drugih autora. Na modelima koje smo kasnije razvili, mi smo uspjeli potpuno imitirati primjerice morbus Wilson, a dijelom i choreu minor. To je bio razlog zašto smo smatrali potrebnim da se organizira jedan međunarodni simpozij o eksperimentalnom tremoru. Odaziv vrlo uglednih stručnjaka sa tog polja iz inostranstva i naše zemlje koji su došli u Sarajevo, dokaz je po mom mišljenju da je sazrela situacija za takav sastanak. Naši radovi su osim nastojanja pronalaženja adekvatnih modela bili usmjereni i na probleme metabolizma bolesti striatuma specijalno metabolizma željeza i bakra. Iako je to jedan novi parametar, on je ipak u najužoj vezi sa već poznatim promjenama metabolizma dopamina, pa se nadamo da će se na taj način proširiti polje interesa za biokemijske promjene koje prate ovu grupu bolesti.

Cilj svih naših nastojanja, svih koji smo ovdje, je u prvom redu pitanje terapije bolesti o kojima ćemo raspravljati. Siguran sam da je i vaša želja kao i moja, da ovaj Simpozijum takođe da svoj prilog terapiji ljudi bolesnih od tih bolesti.

P. ŠTERN

OPENING ADRESS

We have met to discuss about the present day situation of diseases caused by metabolic changes in the striatum and to establish how best these diseases can be examined in experiments in men and animals. Symposiums of this character have not been rare in the last ten years, which firstly speaks for the reality of the theme, and secondly it confirms that in a short time many facts have accumulated which are necessary to examine critically and confront with earlier conceptions. We know that biochemical changes in the striatum which lead to Parkinson's or Wilson's disease, have become accessible and clearer only in the last twelve years. From that time until today, a great deal has been published in this field and in the sense of fundamental investigations on animals, or clinically in man, not a little success in therapy has been made. Such a great progress in knowledge of biochemical changes as in diseases of the striatum, has not up to now been attained in any other group of neurological or psychiatric diseases. This necessarily requests further examination and good models in animals, in order to make possible the continuation of such themes and the intensity of obtaining new information. Thanks to a good model, Prof. Zec and I some years ago, could show for instance, that alpha-methyl-dopa gives a good therapeutic effect, not only in animals but in human pathology in cases of Wilson's disease or chorea minor. This is also confirmed by other workers. On the models we developed afterwards, we succeeded completely to imitate Wilson's disease and partly chorea minor. That was the reason, we considered it necessary to organise an international symposium on experimental tremor. The response of authoritative experts in this field from abroad and in our country who have come to Sarajevo, in my opinion, is proof, that the situation is mature for such a meeting. Our works, apart from efforts to discover adequate models were directed to programme the metabolism of diseases of the striatum, especially the metabolism of iron and copper. Nevertheless it is a new measure, however it is in the narrowest relation with already known changes of metabolism of dopamine, and we hope that in such a way to widen the field of interest in biochemical changes which follows this group of diseases.

The aim of all our efforts, those of us who are here, is in the first place the question of therapy of the disease, about which we shall discuss. I am sure that your wish as well as mine, is that this symposium will also add to the therapy of patients with these diseases.

N. ZEC, HONORABLE PRESIDENT

OPENING ADRESS

Dear colleagues, Ladies, and Gentlmen,

I have the great honour to extend on behalf of the Department of Medical Sciences of our Academy of Science and Arts a cardial welcome to all participants of the Symposium and particularly to those from foreign Countries who accepted kindly our invitation to take active part on this international meeting.

The important discovery of Carlson (1959) and his coworkers that dopamine is present in the central nervous system in the first place in the striatum and the nucleus niger and that this substance is reduced in Parkinson's disease as found by Hornykiewicz and Barbeau lead to the first attempts to treat Parkinson's disease by dopamine (Birckmayer, 1961).

From then up to the present days there is made a great step forward in our knowledge of biochemical changes which are accompanying striatum diseases and in the respective clinical achievements as well as in the great importance of catecholamine in neuro-psychiatry.

We know quite well that the treatment of shizophrenia with various phenothiazines or other compounds is deep influencing the metabolism of catecholamins what relates also to the treatment of depressive conditions by different antidepressive drugs. Therefore investigations on metabolism of mediators (catecholamine, acetylcholine and serotonin) are today an inseparable part of moderne neuro-psychiatry whether in experimental or clinical medicine.

The present international symposium on experimental tremor is actually induced on this account to exchange experiences on biochemical alterations in the Central Nervous System, particularly alterations regarding striatum diseases and on the role of catecholamine in the moderne neuro-psychiatry.

I am convinced that these proceedings will provide a significant contribution to the elucidations of the problem of experimental tremor and please accept my sincere wishes for its success.

Anatomy and methods



E. NAVRATIL

PERSONAL RECOLLECTION ON MY WORK WITH OTTO LOEWI

Vrlo poštovani gospodine predsjedniče!

Na ljubaznoj ponudi gospodina profesora Sterna da i ja učestvujem na ovom internacionalnom Simpozijumu, iako to nije moja specijalnost, ja sam se rado odazvao i dopustio sebi da oživim sjećanja iz mog djetinjstva i ponovo vidim svoj rodni grad. Ja sam, znači, stari Sarajlija. Meni je veoma žao što neću moći na vašem jeziku održati svoje predavanje, pa zato dozvolite mi da to učinim na njemačkom i engleskom jeziku.

Die fast 50 Jahre, die seit dem Beginn meiner wissenschaftlichen Arbeit verstrichen sind, haben begreiflicherweise manche Erinnerung durch neue Eindrücke überlagert. Seit ich jedoch die Einladung erhielt, über meine Zeit mit Otto Loewi zu sprechen, steht sie ganz lebendig und gegenwartsnahe vor mir, als ob nicht fast ein halbes Jahrhundert vergangen wäre.

Im Jahre 1921 begann ich an der Karl-Franzens-Universität in Graz mit dem Medizinstudium. Bereits zwei Jahre später trat ich in den

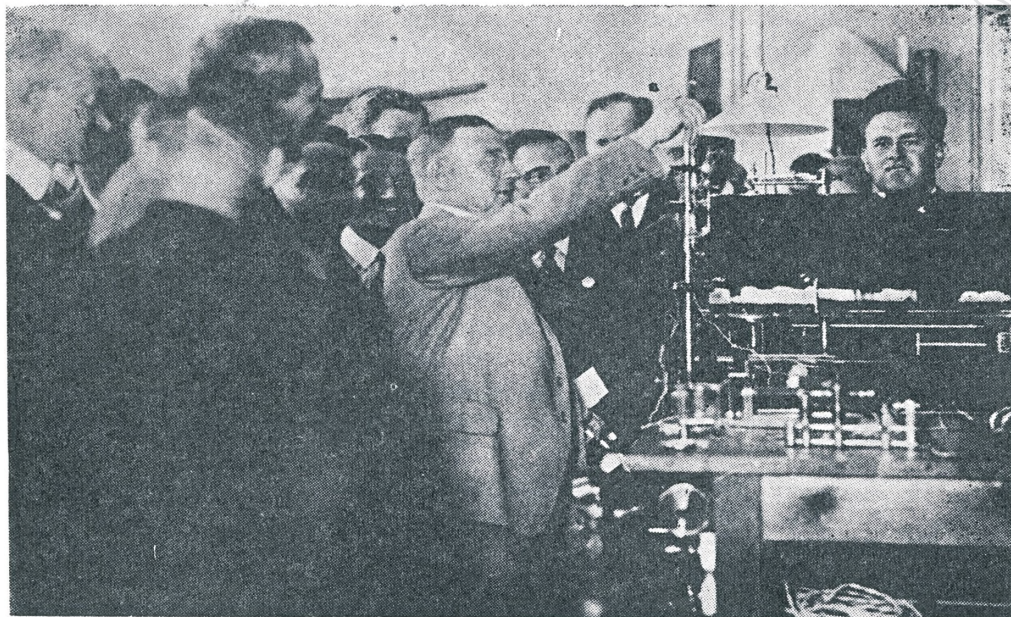


Fig. 1

Verband des Institutes für Experimentelle Pathologie, dem Prof. Pfeiffer vorstand, als Mitarbeiter seines Assistenten Dr. Ernst Kupelwieser ein. Kupelwieser war ein exakter Forscher, dessen besondere Vorliebe der Mathematik galt. Er beschäftigte sich damals mit dem auch heute aktuellen Problem der Nachweisbarkeit immunisatorisch bedingter Fermentprozesse. Aus dieser Zeit stammen zwei in den Jahren 1925 und 1926 in der Biochemischen Zeitschrift erschienene gemeinsame Veröffentlichungen, die das angeführte Thema behandelten. Als Kupelwieser eine Assistentenstelle am Physiologischen Institut in Wien übernahm, empfahl er mich dem Vorstand des Pharmakologischen Institutes Hofrat Prof. Otto Loewi für eine vakante Hilfsassistentenstelle und so trat ich am 1. 4. 1924 meinen Dienst an diesem Institut an, das ich erst nach meiner Promotion am 30. 9. 1927 verlassen sollte. Bei meinem Eintritt in das Institut hatte Otto Loewi bereits im Jahre 1921 die aufsehenerregende 1. Mitteilung über humorale »Übertragbarkeit der Herznervenwirkung« in Pflügers Archiv für die gesamte Physiologie des Menschen und der Tiere veröffentlicht, ebenso war schon die 2. Mitteilung im Jahre 1922, die 3., 4. und 5. im Jahre 1924 in dieser Zeitschrift von ihm veröffentlicht worden. Mit der Durchführung der von Otto Loewi beabsichtigten experimentellen Untersuchungen wurde ich gleich vertraut. Als erstes lernte ich die Präparation des Froschherzens mit und ohne der Herznerven. Otto Loewis Stolz war ein grosses, von der Rockefeller Foundation gestiftetes Kymographion, dessen Getriebe mit der Hand zu betätigen war. Seine Bedienung war daher schwierig. Ausser dem Kymographion besass das Institut noch eine Zentrifuge und Glasgeräte. Andere Apparate gab es nicht. Mein ganzes Bestreben galt einer genauen Registrierung der Herztätigkeit. Inwieweit mir dies gelang, kann nach den in den späteren Veröffentlichungen wiedergegebenen Abbildungen beurteilt werden.

Bald nach meinem Eintritt in das Institut erzählte mir Otto Loewi, wie er zu seiner Entdeckung gekommen war. Ich entnehme diese Beschreibung seiner autobiografischen Skizze aus dem Buche »Otto Loewi« von F. Lembeck und W. Giere und darf die betreffende Stelle wortwörtlich wiedergeben:

»As far back as 1903, I discussed with Walter M. Fletcher from Cambridge, England, then an associate in Marburg, the fact that certain drugs mimic the augmentary as well as the inhibitory effects of the stimulation of sympathetic and/or parasympathetic nerves on their effector organs. During this discussion, the idea occurred to me that the terminals of those nerves might contain chemicals, that stimulation might liberate them from the nerve terminals, and that these chemicals might in turn transmit the nervous impulse to their respective effector organs. At that time I did not see a way to prove the correctness of this hunch, and it entirely slipped my conscious memory until it emerged again in 1920.

The night before Easter Sunday of that year I awoke, turned on the light, and jotted down a few notes on a tiny slip of thin paper. Then I fell asleep again. It occurred to me at six o'clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night, at three o'clock, the idea returned. It was the design of an experiment to determine

whether or not the hypothesis of chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design. I have to describe briefly this experiment since its results became the foundation of the theory of chemical transmission of the nervous impulse.

The hearts of two frogs were isolated, the first with its nerves, the second without. Both hearts were attached to Straub cannulas filled with a little Ringer solution. The vagus nerve of the first heart was stimulated for a few minutes. Then the Ringer solution that had been in the first heart during the stimulation of the vagus was transferred to the second heart. It slowed and its beats diminished just as if its vagus had been stimulated. Similarly, when the accelerator nerve was stimulated and the Ringer from this period transferred, the second heart speeded up and its beats increased. These results unequivocally proved that the nerves do not influence the heart directly but liberate from their terminals specific chemical substances which, in their turn, cause the well-known modifications of the function of the heart characteristic of the stimulation of its nerves.

The story of this discovery shows that an idea may sleep for decades in the unconscious mind and then suddenly return. Further, it indicates that we should sometimes trust a sudden intuition without too much skepticism. If carefully considered in the daytime, I would undoubtedly have rejected the kind of experiment I performed. It would have seemed likely that any transmitting agent released by a nervous impulse would be in an amount just sufficient to influence the effector organ. It would seem improbable that an excess that could be detected would escape into the fluid which filled the heart. It was good fortune that at the moment of the hunch I did not think but acted immediately.

Loewi kam regelmässig zwischen 8 und 8.30 Uhr in das Institut. Die tägliche Arbeit begann mit einer eingehenden Besprechung mit seinen Mitarbeitern. Nach 1 Uhr ging er zum Essen in seine nahegelegene Wohnung Johann Fuxgasse 35 und war bereits nach 2 Uhr wieder im Institut, um seine Vorlesung vorzubereiten. Gegen 8 Uhr abends wurde es noch später. Das Mittagessen nahm ich in einem kleinen Gasthaus ein, das das Stammlokal des Nobelpreisträgers Fritz Pregl war. Das Beisammensein mit ihm war für mich als jungen Studenten begreiflicherweise ausserordentlich anregend.

In der kultivierten und vornehmen Wohnung Loewis fanden regelmässige Jours statt, an denen Wissenschaftler und vor allem bekannte Musiker teilnahmen. Sehr bald wurde auch ich eingeladen und war bei Loewis wie zu Hause. Bei dieser Gelegenheit erinnere ich mich an eine kleine Episode: Eines nachmittags wollte ich mich vorzeitig von Hofrat Loewi verabschieden mit der Begründung, ich sei am Abend eingeladen. Auf seine Frage: »wo?« antwortete ich: »bei Frau Hofrat Loewi«, worauf er mir sagte: »Schauen Sie, Navratil, dort ist es meistens so fad, bleiben Sie lieber mit mir im Institut, wir werden dann später gemeinsam zu Hause nachtmahlen.« Mit seinen drei Söhnen, die wesentlich jünger waren als ich, war ich sehr befreundet, eine Freundschaft, die nie ganz abgerissen ist, besonders nicht mit Viktor, den ich anlässlich meiner Besuche in Buenos Aires immer wieder traf.

Im Jahre 1926 demonstrierte Otto Loewi anlässlich des XII. Internationalen Physiologenkongresses in Stockholm den Übertragungsversuch und seine Beeinflussung durch Physostigmin. Die Fotografie, die damals aufgenommen wurde und die er mir schenkte, gibt die Durchführung des Versuches wieder (Abb.). Die grundlegenden Experimente wurden ausserdem zahlreichen in- und ausländischen Gästen im Institute vorgeführt, unter anderen hatte ich den Vorzug, sie auch dem Altmeister der Pharmakologie Hans-Horst Meyer vorzuführen. Bedauerlicherweise gelingt, wie Sie ja wissen, nicht immer ein Versuch. Da sich das Glück nicht erzwingen lässt, lud mich Otto Loewi an solchen Nachmittagen zu einem Spaziergang ein, wobei nicht nur wissenschaftliche, sondern auch allgemeine Fragen besprochen wurden. Welch ein Gewinn dieser enge Kontakt für mich war, brauche ich Ihnen wohl nicht eigens zu sagen. Bei der wissenschaftlichen Vielseitigkeit Otto Loewis ist es verständlich, dass zeitweise sein Interesse an der humoralen Übertragbarkeit dadurch nachliess, dass er sich mit anderen Ideen befasste z. B. mit dem Problem der Wirkungsweise des Insulins. In dieser Zeit blieb ich mir mehr oder weniger selbst überlassen. Dass Otto Loewi gemeinsam mit Sir Henry Dale im Jahre 1936 der Nobelpreis für Physiologie und Medizin zuerkannt wurde und zwar für die Entdeckungen in bezug auf die chemische Übertragung der Nervenwirkung, ist Ihnen ja wohl bekannt. Für mich war es natürlich eine besondere Freude, dass ich in seinem Nobelvortrag, gehalten in Stockholm am 12. Dezember 1936, der den Titel »Die chemische Übertragung der Nervenwirkung« trug, unter seinen Mitarbeitern an erster Stelle von meinem grossen und unvergesslichen Lehrer genannt wurde.

Rückblickend kann ich sagen, dass die Jahre, die ich unter seiner Leitung im Pharmakologischen Institute verbrachte, in jeder Beziehung zu den schönsten meines Lebens gehörten. Ich war jung, von Otto Loewi fasziniert und von dem erschlossenen wissenschaftlichen Neuland begeistert. Diese Jahre waren ausschlaggebend für meine weitere wissenschaftliche Entwicklung.

Nach seiner Emigration nach New York habe ich Otto Loewi wiederholt besucht und mit ihm bei diesen Gelegenheiten über diese wunderbare Zeit gesprochen. Als er mir sein Buch »From the Workshop of Discoveries« im Jahre 1953 sandte, widmete er es mir daher mit vollem Recht mit den Worten: »Ernst Navratil — Zur Erinnerung an die schönste Zeit seines Lebens«.

Ja se vama puno zahvaljujem moje dame i gospodo za moje održano predavanje i hvala vam na vašoj pažnji.

E. NAVRATIL

LIČNA SJECANJA NA MOJ RAD SA OTTOM LOEWIEM

KRATAK SADRŽAJ

Autor je počeo još kao student da radi u laboratoriju nobelovca prof. Loewia, čovjeka koji je prvi eksperimentalno dokazao humoralnu transmisiju podražaja. Mnogi važni radovi sa toga područja su zajedno publicirani. U ovom referatu prikazan je Otto Loewi i kao čovjek i kao učenjak — kako ga je vidio njegov najbliži suradnik.

H. HADZISELIMOVIC

ASPECT OF THE EXTRAPYRAMIDAL NUCLEI IN RELATION TO THE CONFIGURATION OF THE HUMAN SKULL

Studying the appearance of the brain structures in relation to the configuration of the skull the appearance and the position of the extrapyramidal nuclei have been found by the author to depend on the type of brain resp. on the type of skull. According to various authors the extrapyramidal nuclei are formed by n. caudatus, putamen, globus pallidus, n. subthalamicus, n. ruber and n. niger. According to Spatz a closed functional apparatus, termed extrapyramidal motor system, is formed by the motor nuclei of the tectum, n. dentatus cerebelli, n. ruber, n. niger, n. subthalamicus, at last thalamus opticus, globus pallidus and the large ganglion masses of corpus striatum.

I was studying the asymmetry of the nuclei of the extrapyramidal system in relation to the asymmetry of the brain, i. e. in relation to the occipital, temporo-parietal and frontal portion of the skull.

Various authors were dealing with the asymmetry of the brain: Elliot-Smith (1907), Shindo (1913), Förtig (1922), Minkin (1929), Inglessis (1925), Karvé (1931), Ikeda (1931), Werner (1958), Hadžiselimović and Andjelić (1963) et al. Elliot-Smith (1907) established occipitopetalia on the left side in 80% of cases. Marked occipitopetalia is characteristic of Egyptian skulls. Förtig (1922) investigated lateral ventricles in 217 psychopaths. The left ventricle was found by Förtig to be larger than the right in 66.4% and smaller in only 17.5% of cases. In 77.8% of cases the left ventricle was wider than the right and more markedly retracted backward. The same asymmetry was established by the author in 28 normal individuals. The author considers the larger volume of the left hemisphere to be due to the functional superiority of the same hemisphere and this superiority to be expressed by an increased production of liquor.

MATERIAL AND METHODS

By measurement of the oblique skull diameters I determined the type of skull as well as the type of brain conform to it. The material used consisted of 500 randomized macerated and degreased skulls. On 250 brains, especially preserved, anatomical preparations were elaborated by careful dissection. On these preparations the appearance and

position of the extrapyramidal nuclei in relation to the configuration of the brain and type of skull were studied by the author.

RESULTS OF INVESTIGATIONS

ON 500 randomized macerated and degreased human skulls, I established by measurement of the oblique diameters entirely symmetrical skulls merely in 2 per cent. of the investigated cases. In 42 per cent. of the investigated cases I found occipital symmetry in association with temporo-parietopetalia either of the right or the left side. This temporo-parietopetalia may be associated with the frontopetalia either of the same or of the opposite side. Cases were found in which the occipital symmetry was associated only with the frontopetalia either of the right or left side as well (1.6 per cent).

In 36.8 per cent. of the investigated cases left occipitopetalia in conjunction with temporo-parietopetalia and in certain cases with frontopetalia either of the opposite or of the same side of the skull were established as well.

In 19.2 per cent. of the investigated cases I established right occipitopetalia either by itself or in association with prominences of the temporo-parietal and frontal portion either of the left or right side of the skull.

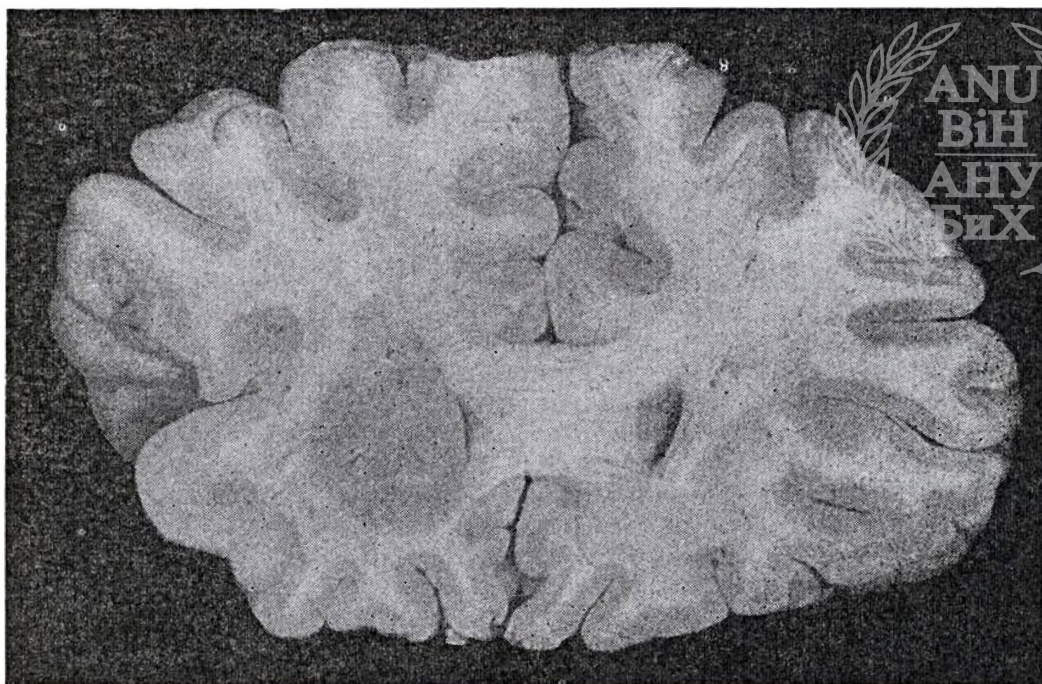


Fig. 1.

In the cases of occipital symmetry of the brain in association with temporo-parietopetalia of the right or left side, the extrapyramidal nuclei are situated laterally on the side of temporo-parietopetalia (42 per cent.).

In the cases of left occipitopetalia (36.8 per cent.) in association with right temporo-parietopetalia and right frontopetalia the extrapyramidal nuclei are retracted backward on the side of occipitopetalia. These nuclei are in antero-lateral position on the side of frontopetalia and temporo-parietopetalia. In the cases of right occipitopetalia (OPD, 19.2 per cent.) in association with left frontopetalia (FPS), the extrapyramidal nuclei are retracted backward on the right side and are situated antero-laterally on the left side.

On the frontal section through genu corporis callosi (Fig. 1) the antero-lateral position of n. caudatus on the left side i. e. on the side of frontopetalia and temporo-parietopetalia, is manifest. On the right side, on the side of occipitopetalia n. caudatus is not apparent. On the section through the anterior portion of corpus striatum (Fig. 2) the left



Fig. 2

caput of n. caudatus appears to be in an anterior position. On the left side i. e. on the side of temporo-parietopetalia globus pallidus which is missing on the side of occipitopetalia, is evident. On the right side, i. e. on the side of occipitopetalia on which all brain structures are retracted backward, n. caudatus and putamen are joined by their lower portions so that the internal capsule separates them incompletely. On the left side i. e. on the side of temporo-parietopetalia caput n. caudati is separated from putamen and globus pallidus by means of capsula interna and on that side the posterior portion of the anterior brain commissure is distinctly marked. On the frontal section through chiasma opticum (Fig. 3) n. lentiformis is more pronounced on the side of occipi-



Fig. 3

topetalia i. e. on the right side. On the frontal section through corpora mamillaria (Fig. 4) on the left side i. e. on the side of temporo-parieto-petalia and frontopetalia, n. subthalamicus, n. ruber and n. niger are



Fig. 4

more pronounced than on the side of occipitopetalia. On the right side i. e. on the side of occipitopetalia n. lentiformis and claustrum are well marked and on this side corpus amygdaloidum appears distinctly pronounced. On the horizontal section (Fig. 5) in the cases of left occipito-

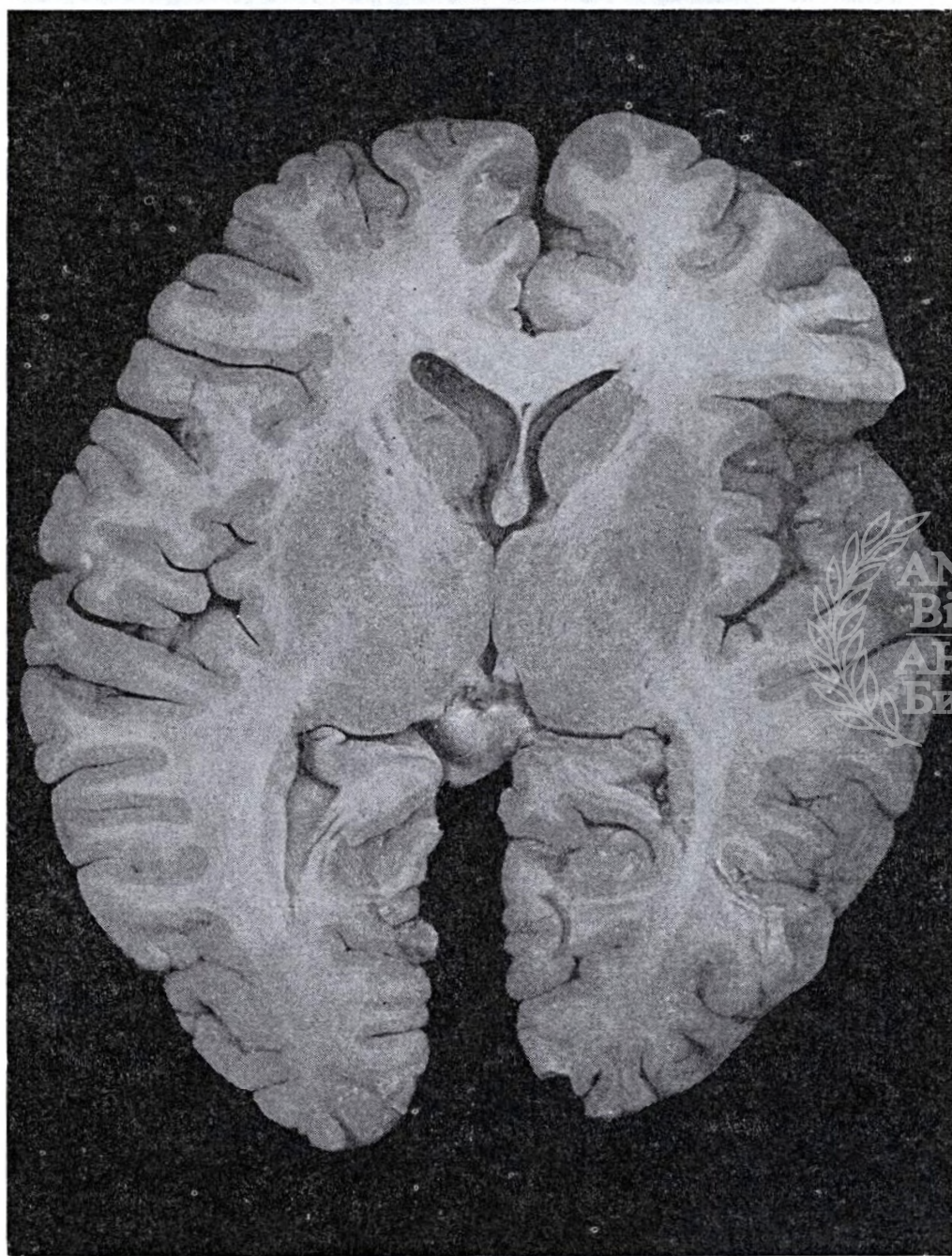


Fig. 5

petalia in association with right temporo-parietopetalia and right frontopetalia, n. caudatus and putamen are found to be in an antero-lateral position on the right side.



Fig. 6.

In the cases of right occipitopetalia in conjunction with left frontopetalia and left temporo-parietopetalia (Fig. 6) n. caudatus and putamen

are in an antero-lateral position on the left side. On an especially elaborated brain preparation (Fig. 7) n. ruber, n. niger, and n. dentatus are clearly perceptible to be in an antero-lateral position on the right side i. e. on the side of frontopetalia and temporo-parietopetalia.

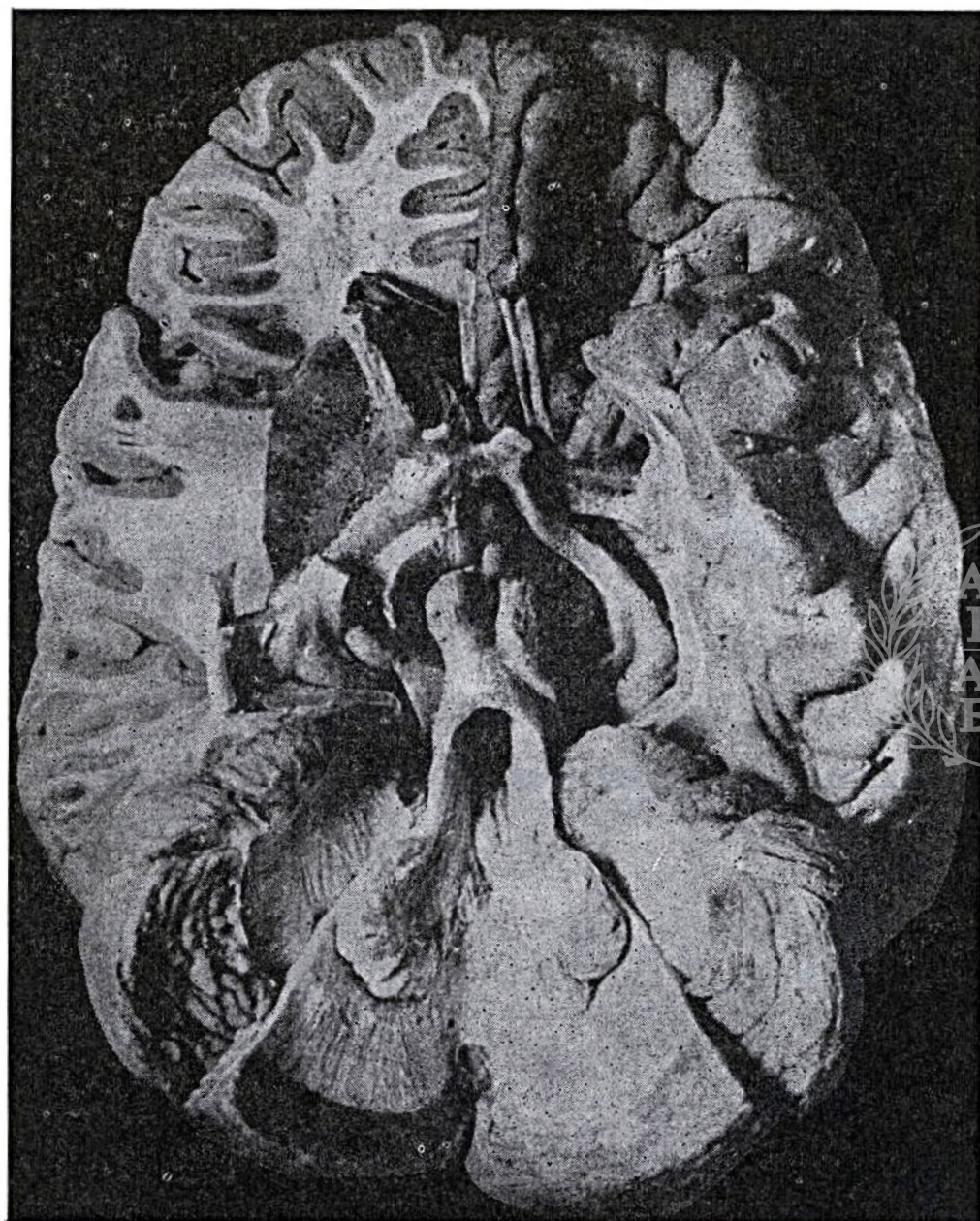


Fig. 7.

The appearance and position of the extrapyramidal nuclei depend on the configuration of the brain and the type of skull. Asymmetry of

the extrapyramidal nuclei is readily apparent if the type of the brain is determined by measurement of oblique diameters of a corresponding skull.

On live individuals the type of skull and that of brain can be determined by measurement of oblique diameters. Owing to the type of brain the appearance and position of extrapyramidal nuclei of investigated individuals are determined.

SUMMARY

On 500 randomized macerated and degreased human skulls the type of skull and type of brain conform to it were determined by measurement of oblique diameters owing to established points. On 250 brains especially preserved anatomical preparations were worked out by careful dissection. On frontal and horizontal brain sections as well as on especially elaborated preparations, the appearance and position of the extrapyramidal nuclei in symmetrical and asymmetrical brains were investigated. On the side of frontopetalia and temporo-parietopetalia the investigated extrapyramidal nuclei are in an antero-lateral position, whereas those on the side of occipitopetalia are retracted backward. Owing to the type of skull and to the type of brain the appearance and position of the extrapyramidal nuclei of the investigated individual may be determined.

H. HADŽISELIMOVIC

IZGLED EKSTRAPIRAMIDALNIH JEZGARA U ODNOSU NA KONFIGURACIJU ČOVJEKOVE LOBANJE

KRATAK SADRŽAJ

Mjerenjem kosih promjera na 500 čovječjih lobanja, uzetih bez izbora, određen je tip lobanje. U 2% ispitivanih slučajeva radilo se o potpuno simetričnim lobanjama. U 42% ispitivanih slučajeva konstatovalo sam okcipitalnu simetriju udruženu sa temporo-parietopetalijom desne ili lijeve strane. U 36,8% ispitivanih slučajeva radilo se o okcipitopetaliji sa lijeve strane, koja je bila kombinovana sa desnom temporo-parietopetalijom, a u nekim slučajevima i sa desnom frontopetalijom. U 19,2% ispitivanih slučajeva ustanovio sam desnu okcipitopetaliju udruženu sa lijevom temporo-parietopetalijom, a u nekim slučajevima i sa lijevom frontopetalijom.

Na 250 specijalno konzerviranih i prepariranih mozgova ispitivao sam izgled unutrašnjih moždanih struktura kod simetričnih i asimetričnih mozgova. Mjerenjem kosih promjera odredio sam prvo tip lobanje, a zatim izvadio mozak iz lobanje i odredio njegov tip. Na frontalnim i horizontalnim presjecima ispitivao sam izgled i položaj n. caudatus-a,

putamen-a, globus pallidus-a, n. subthalamicus-a, thalamus opticus-a, substantiae nigrae, n. ruber-a i n. dentatus-a kod simetričnih i asimetričnih mozгова. Na specijalno izrađenim moždanim preparatima proučavao sam izgled i položaj ekstrapiramidnih jezgara. Na strani okcipitopetalije ekstrapiramidne jezgre su povučene prema nazad, a na strani temporo-parietopetalije i frontopetalije te su jezgre latero-i anteroponirane. Moguće je na živim osobama, mjerenjem kosih promjera lobanje, odrediti tip lobanje i mozga i prema tome suditi o izgledu i položaju ekstrapiramidnih jezgara i ostalih unutrašnjih moždanih struktura ispitivane osobe.

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DISCUSSION

Schnieden: 1) Is it possible that fixation artefacts could have accounted for some of the results you observed in the brains you examiend? 2) It would appear your results on asymetry might be of help to the stereotaxic surgeon. Have you measured oblique radius of skull at post mortem, tried to place a stereotaxic lesion and then confirmed correctness of lesion placement by checking the brain?

Hadžiselimović: 1) I investigated only the macerated and degreased skulls and preserved brains. I have no experience on the living persons, especially by stereotaxic operations. 2) That is not artefact, all structures are normal. My results are possible ro examine on the living individuals.

NEDŽAD GLUHBEGOVIC

COMPARATIVE ANATOMICAL STUDY OF THE CORPUS STRIATUM

(Professional work)

As Johnston, Herrick and Obenchaim have demonstrated, striatal region of cyclostomes is partly invaginated in the hemisphere. Johnston describes the fibres, which originate from the striatal area to dorsal thalamus and probably to mesencephalon. He did not find the hypothalamic connections.

According to Holmgren and Jansen, basal area in the brain of myxinoids lies in the ventral part of forebrain. They consider part of this area homologous with the corpus striatum of higher forms. It is however yet to be discussed, but there is no doubt that in the telencephalon of myxinoids dominate primary, secondary and, to some extent, tertiary olfactory connections.

In plagiostomes part of striothalamic tract and striohypothalamic tract, which originate from striatum, can be compared with somatic projecting tract of Johnston. It terminates in the ventral thalamus and Johnston suggested that these fibres can represent the beginning of the pyramidal tract. According to Huber and Crosby it is most probably that those fibres are forerunner of the ansa lenticularis of higher forms. Johnston has described the fibres, which connect the striatal area of one side with that of the opposite side and he considered them to be homologous to the corpus callosum.

In ganoids and teleosts Sheldon described an area within telencephalon, which is partly under the influence of the olfactory system and is partly supplied with somatic fibres, to which he gave the term paleostriatum. The term paleostriatum is used to describe the specific areas of the reptilian and avian brain. There exists a reason for believing that part of these areas in reptiles (paleostriatum augmentatum) can be compared with paleostriatal area in teleosts, but in the absence of a direct proof for it, we use the term olfactosomatic area, which is given by Herrick. This area is connected with the hypothalamic and certain thalamic areas of diencephalon.

Ventrolateral quadrant of the amphibian hemisphere contains primordium striati and amygdaloid complex (archistriatum or sometimes

epistriatum of higher forms). Herrick has divided the striatal part of this area in dorsal, ventral part and caudate nucleus, with its medial extension *n. accumbens*. It seems that *n. caudatus* of these forms is probably homologous, at least partly, with the head of the caudate nucleus of higher forms, but it is not possible to find out the real homology between dorsal and ventral striatal areas in one hand and various nuclei (*globus pallidus*, *putamen* and the tail of caudate nucleus) on the other hand in higher forms. Descending pathways of striatal area are mainly homologous with those of lower forms, but here they are better separated and terminate in thalamic and hypothalamic regions. Of special importance are the striopallial connections, which are described by Herrick and which connect dorsal part of the striatum with *primordium pallii*, representing the important tract for impulses, regarding to the development of pallium.

Telencephalon of reptiles differs from that of lower vertebrates by greater structural differentiation and increase of its size in the relation to the size of the other parts of the brain. However there exists a difference in the development of various reptiles. The telencephalon of *sphenodon* and turtle is more primitive than that of the snake. Telencephalon of lizard is to some extent similar to that of aplacental mammals (*monotremata* and *marsupialia*), whereas on the other side it is similar to the telencephalon of crocodiles and birds. The telencephalon of crocodiles can be compared with that of birds. In reptiles the striatal complex is highly developed. Even in lower reptiles, as in turtle, the increasing size of this region is remarkable, if it is compared with that of amphibians. Johnston has subdivided the striatal area of turtle in caudate nucleus, lentiform nucleus and dorsal ventricular ridge. The medial extension of caudate nucleus is usually considered as *n. accumbens*. On the dorsal and dorsolateral side of caudate nucleus lies lentiform nucleus. Earlier Johnston had considered the dorsal ventricular ridge as somatic as a whole and showed its connections with pallial areas, to which he gave the term general pallium. That indicates the great development of the somatic area in the lateral wall of the hemisphere. Later Johnston had shown that the dorsal ventricular ridge of the turtle appears as a thickening of the lateral border of pallium and that its continuous growth causes it to project to the ventricle.

Smith in the study of corpus striatum of *sphenodon*, has subdivided the lateral hemisphere wall in paleostriatal and hypopallial areas. According to him paleostriatal part is homologous to *globus pallidus* of the mammals. He considered the hypopallial region as a representative of the greater part of caudate nucleus and putamen. He gave a special importance to the relations of the lateral striate artery and he confirms that »if the relations of the lateral striate artery be compared in a series of reptilian and mammalian brains they afford a striking corroboration to the hypothesis that a large part of the mammalian corpus striatum is homologous with the reptilian hypopallium and is therefore cortical in origin«. Cairney has subdivided the striatal areas, except *archistriatum*, in *olfactostriatum*, *somatic striatum* and *hypopallium*. *Olfactostriatum* and *somatic striatum* he considers together as paleostriatal. *Hypopallium anterius*, which is continuous with the general cortex, Cairney

considered as striatal, and above all neostriatal. Hypopallial ridge as a whole can be compared with the dorsal ventricular ridge in turtle and its caudal part Cairney and Johnston consider as amygdaloidal. As it is seen from this it is difficult, because of the differences in relationships in various reptiles as well as in variations in terminology, to correlate the results of different investigators in order to get the basic plan or pattern of the striatal part of reptilian hemisphere. Striatal region of reptiles is connected by means of corresponding tracts with the thalamic, hypothalamic and mesencephalic regions.

The paper of Edinger, Wallenberg and Holmes of the year 1903 marks the beginning of modern investigations of avian telencephalon. And Kalisher and Rose dealt with these investigations. Huber and Crosby have shown the nuclear configuration of the lateral wall of the sparrow's telencephalon, with temporary comparisons with the conditions in other birds. These centers can be subdivided in *n. basalis*, *paleostriatum* with its two parts- *paleostriatum primitivum* and *paleostriatum augmentatum*, *ectostriatum*, *neostriatum*, *hypostriatum*, *n. intercalatus hyperstriati*, *hyperstriatum accessorium*, *archistriatum* and *n. teniae*. Johnston had shown that the area, which is equivalent to *paleostriatum augmentatum* and to its extension *n. accumbens*, develops in mammals in the head of caudate nucleus and it can form a smaller part of putamen, as Kappers has shown. *Paleostriatum primitivum* is probably the forerunner of *globus pallidus* in mammals. Johnston has shown that *neostriatum*, which is much better developed in birds than in reptiles, represents putamen in mammals. In mammals the development goes in the direction of the forming of superficial layers, which fold and form the gyri and sulci. In reptiles and birds the development goes together with thickening of the wall, preceded by the thickening of periventricular gray. *Hyperstriatal* area of birds acts to certain extent in a similar way as the cortical area of some lower mammals. In sparrow, pigeon and duck accessory *hyperstriatal* area is relatively well developed, as it is seen from its size and from its fibre connections. In the majority of avian brains olfactory system is reduced, although exceptions exist. Somatic center of the hemisphere have grown, which represents further differentiation in relation to reptiles. This differentiation goes together with the increase of the dorsal thalamus differentiation in birds, compared to reptiles.

Basal nuclei of the mammalian hemisphere include amygdaloid complex, *n. caudatus* and *lentiformis*, *claustrum* and certain gray which join these big nuclear groups.

Amygdaloid nuclei (*archistriatum*) were subjects of investigation of many observers. 1896 von Kölliker discovered four nuclei in the basal part of the hemisphere of the rabbit, which belong to the amygdaloid group, but he gave the term *n. amygdale* to only one of them. Wölsch, working on monkeys, has identified seven nuclear groups of *archistriatal* complex. De Vries found four nuclei within this complex. Johnston and Humphrey subdivided the nuclei of amygdaloid complex in one group, which is more primitive in its development and which originates from olfactory area of lower forms and which is of basal

origin, and another group, of cortical origin, which appears later in phylogenetical history. The relations of the amygdaloid complex differ according to the changes of general morphology of telencephalon.

N. caudatus and n. lentiformis form clear and vivid mass in the mammalian brain. Lentiform nucleus is usually divided into medial part — globus pallidus and lateral nucleus — putamen. According to Kappers putamen, together with caudate nucleus form the main mass of neostriatum. However part of n. caudatus is considered as paleostriatal by origin. Dividing the caudate-putamen complex in two parts by internal capsule is a secondary consideration and it is not the same in all mammals. Paleostriatum is phylogenetically older, as we see from its earlier ontogenetical differentiation. According to Johnston, the caudate nucleus has two origins, consisting phylogenetically of a ventral and a dorsal part. The ventral part or olfactostriatum, Cairney considered as a reptilian homologue for a part of the head of the caudate nucleus. Johnston considered the rest of the head and part of the tail as homologous to the dorsal ventricular ridge of the turtle. So, in caudate nucleus we can recognize, according to its origin, subpallial part, which is olfactive in relation to its type of fibre connections and the pallial part, which is closely related to the cortex. Putamen, as Johnston has found, is also pallial by origin, but its development is faster than that of caudate nucleus. Generally it is accepted that globus pallidus is phylogenetically old and has its representative in paleostriatum primitivum (or somatic area) lower forms.

Fibre connections of the corpus striatum in mammals are divided in thalamic connections, internuclear connections, efferent pathways to lower centers and cortical connections. Many authors have described the connections of the medial thalamic nucleus with corpus striatum. Von Monakow believed that even the connections between the anterior thalamic nuclei with c. striatum exist. Internuclear connections among various parts of corpus striatum are relatively numerous. Lateral and medial medullary lamina, which separates globus pallidus from putamen and medial from lateral parts of globus pallidus, consist of internuclear fibres, supplemented with passing fibres. The main efferent paths of the corpus striatum are elements of the ansa lenticularis. These were studied by many investigators (von Monakow, Wallenberg, Economo, Kleist, C. and O. Vogt, Bickel, Pollak, Riese, Jakob, Morgan, Kodama and others). These fibres terminate in Forel's nucleus, in tegmentum of mesencephalon, in red nucleus, in substantia nigra and in hypothalamic nuclei. Muskens showed the connection between corpus striatum and nucleus interstitialis and Jakob had found striofugal fibres, which terminate in the nucleus of Darkschewitsch. Riese has found that these fibres join the medial longitudinal tract. The caudate-lenticular complex of mammals is homologous to the neostriatal and paleostriatal areas of lower forms, and ansa lenticularis is the representative of efferent components of lateral forebrain bundle of these lower forms. Tilney and Riley describe that in fish within the corpus striatum lies the highest correlating centre for motor impulses and that swimming movements represent the main motor activity of the body. These movements are characterized by rhythmic continuity and automatic association. In course

of evolution, when terrestrial life became the order of the day, these constant movements became useless and the neostriatal areas developed as inhibitors of activity of primitive paleostriatal areas and so at last caudate - lenticular complex of lower forms developed.

One of the problems which interests the investigators is the relation between the cortex and the caudate - lenticular complex. Ramon y Cajal, Déjérine, Economo and others pointed out the connections of nucleus caudatus with the cortex, through internal capsule. However, there are authors who insist that these connections do not exist or that they are very poorly developed (Wilson, Jakob). The proofs of the present time show the existence of these connections.

Globus pallidus is terminal station of the influences from corpus striatum. Neuron chains of the internal part of globus pallidus, which is under the influence of caudate nucleus and putamen, represent important afferent paths for cortical areas. These cortical areas are the main source of pyramidal tract. In that way this part of extrapyramidal system becomes an afferent path for pyramidal tract. Striatal system depends on cortical areas and used the pyramidal tracts as efferent pathways, so that the pyramidal tract can be considered as part of the mechanism of extrapyramidal activities.

The different morphological development of the extrapyramidal structures in man and animals, the development of the up-right posture, the prevailing of the pyramidal system in man and the different order of the motor mechanism for keeping certain position, are factors at least partly responsible for the differences which exist between the results of experiments on animals and neurological diseases in man. Characteristic appearances of human extrapyramidal diseases are the different hyperkinetic syndroms, which are unknown in animals or they appear rarely. According to Hess the antagonistic »tonic forces« of the central motor regulating systems are normally in the balance, although they are in the latent movement. Visible movements appear only after the change of that balance. Unbalance, caused by inhibition or loss of one or more balanced forces of the nervous system, can result unwilling movements. Much more differentiated balance of motor functions in man, including manipulation with hands and regulation of the up-right position, make them liable to diseases, which appear in the form of unwilling movements.

NEDŽAD GLUHBEGOVIĆ

KOMPARATIVNO-ANATOMSKA STUDIJA KORPUS STRIATUMA KRATAK SADRŽAJ

Komparativno-anatomskom studijom striatalnih predjela različitih vrsta vertebreata moguće je izvesti vrlo interesantan zaključak u pogledu mjesta, izgleda i karaktera njegovih vlaknastih veza.

Striatalne zone vodozemaca pojavljuju se kao receptivni centri uzlaznih talamo-kortikalnih putova i kao eferentne ćelije iz kojih proizlaze vlakna lateralnog prednjomoždane snopa. Amigdaloidni kompleks (ili arhistriatum), manje ili više diferenciran, prisutan je sa svojim karakterističnim vlaknastim vezama.

Striatalne zone reptila pokazuju vidljiv napredak u odnosu na one kod vodozemaca, ali je taj napredak sada ka avian-formama koje nalazimo više ili manje nagoviještene. Izvjesni dijelovi striatalnih zona reptila su homologni s onim u sisara. Može se naglasiti da je cefalična i hipopalična hrptenjača somatska po tipu veza i vjerovatno je predstavnik amigdoloidnih kompleksa sisara.

U većini sličnih mozgova olfaktorni sistem je relativno reduciran. Somatski centri hemisfere su povećani velikim razvojem striatalnih zona.

Ova diferencijacija ide zajedno sa povećanom diferencijacijom dorsalnog talamusa ptica u poređenju sa dorsalnim talamusom reptila.

Bazalni nukleusi hemisfere sisara su nucleus caudatus, nucleus (globus pallidus+putamen) claustrum i nucleus amygdale. Nucleus caudatus i putamen primaju impulse iz talamusa i korteksa, a oni daju eferentna vlakna za globus pallidus i iz tog predjela ansalentikularna vlakna stižu u suptalamičke i srednjomoždane centre, služeći kao centri za združene pokrete. Funkcije kalustruma su neizvjesne. Amigdaloidni kompleks je centar bolje razvijen u nižih sisara nego u čovjeka.

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DISCUSSION

Schnieden: 1) Have you any information on the presence of dopaminergic fibres in striatal areas of reptiles you examined? 2) I was interested in the hypothesis, that in fish, striatal areas are responsible for swimming movements could be used as a piscine model for tremor studies in higher animals.

Gluhbegović: 1) It was emphasized the importance of continual impulses for the movements of swimming of fishes which come from paleostriatum. After their coming out to the shore these movements appeared as disadvantageous and the neostriatum was developed as the inhibitor of these continual impulses from paleostriatum. Maybe, it could be connected with tremor in certain diseases of mans corpus striatum, as well in cases of experimentally provoked tremor of animals.

P. STERN

METHODS FOR CAUSING DISEASES OF THE EXTRAPYRAMIDAL SYSTEM

Six years ago in a paper at the symposium »Methods in drug evaluation«, I presented in a short form, methods of examining antitremogenic substances (AT) (51). Even that analysis showed that there are good possibilities for different forms of tremor (T), rest-tremor (RT), parkinsonic type, non-parkinsonic type and intentional tremor (IT) caused by different drugs, to occur. Besides this we draw attention to spontaneous tremor in animals as well as to the possibility of examination of AT in man. In that paper I was limited to examination of AT, but not to the arrest of symptoms connected with diseases of the striatum, like rigor, and other forms of akinesia, e. g. hyperkinesia of a choriatic character or other kind. As we have developed new methods in the meantime e. g. causing IT Wilson's type (56, 57, 31), and chorea minor (55) and as in our Institute we work intensily on the problem of models of rigor and certain metabolic processes connected with rigor (43, 40, 60), we think that it would be necessary to return again to this theme and to extend it to specific areas. Besides this many other authors have in the meantime published new methods for causing tremor or akinesia. In this paper we shall take into consideration the central causes of T but not to secondary ones e. g. shaking due to the cold etc.

We make a division based on clinical criteria. The methods of causing ST parkinsonic type and ST non-parkinsonic type i. e. those that do not react to known anti-parkinsonics, are shown. Only the methods of causing ST parkinsonic type where the action of oxotremorine (OT) is increased are shown. In this paper in contrast to the one published six years ago (51), we have also presented methods for causing parkinsonic and non-parkinsonic type rigor as well as of forced movements which are similar to chorea and therefore also a disease of the striatum. According to our experiments up to now it seems that the most suitable criteria for parkinsonic type rigor is if it reacts to gamma-hydroxybutyrate (43, 60). With this we could limit the kind of rigor to parkinsonic or non-parkinsonic type.

We will also present methods for causing IT Wilson's type e. g. those which do not react to penicillamine or alfa-methyl dopa and non-Wilson's type (56).

As is seen from the table it is possible to cause forced movements similar to choreatic.

Besides this as in the past paper, the spontaneous tremors which appear in experimental animals and some experimental surgical methods for causing T, are enumerated. Accordingly all the characteristic diseases of the striatum as we find them in human pathology are included in this paper.

TABLES

As we see the possibility of causing tremor or akinesia by pharmacological methods is very large, and so it is necessary to know that the drugs were given i. p., i. m., i. v., or directly into the CNS in cistern or in a certain region, using stereotactic methods. This can be seen from the added tables with reference to different laboratory animals: mouse, rat, rabbit, cat, dog, chicken and monkey.

In experimental medicine it is important to mention the methods caused experimental tremor or rigor in laboratory animals spontaneously or by means of infection. As we have already seen described in dogs, cats, mice and chickens.

The attempts of Rinaldi and Himwich (41) who tried to examine AC in animals by means of EEG, are interesting.

In connection with our presentation it is necessary to mention the collective paper of Jenden (29) which has many useful suggestions as does Friedmann (22).

Finally it is necessary to mention some neuro-surgical methods of causing tremor or rigor but it is immediately necessary to say that opinions for the success of such methods are not of one accord. Jenden (29) explicitly emphasises in his review article that such methods do not always produce the same results. We should like however, to mention the often used method of Vernier and Unna with monkeys, as also the reference article of Aronson et al (4). The surgical method of Carpenter et al. (13) is interesting, where the nuclei of the cerebellum are damaged, and rigor caused by damaging the back hypothalamus.

CONCLUSION

This survey shows that today it is possible to imitate many neurological diseases of the striatum with pharmacological methods. Models like this can surely be used for the discovery of new drugs. For a brilliant example for the investigation of new therapeutic agents on models in laboratory animals we suggest the therapy of Wilson's disease with α -methyl dopa, or chorea with reserpine which we found earlier only on models that we alone constructed.

Table I

EXPERIMENTAL METHODS FOR PRODUCING DISEASES OF STRIATUM

I. Pharmacological methods to produce tremor	< parenteral intracranial
II. Pharmacological methods to produce rigor	< parenteral intracranial
III. Pharmacological methods to produce choreatic movements	
IV. Spontaneous tremor	
V. Surgical methods	
VI. Experimentes on humans	

Table II

TREMOROGENIC SUBSTANCES OF PARKINSONIC TYPE INJECTED PARENTERALLY

	Dosis mg/kg	Animals	References
Tremorine	5—30 i. p.	Mo, d, r, c, m, ch	21
Oxotremorine	0,1—1 i. p. i. v.	Mo, d, r, c, m, ch	14
Acedicline	25 s. c.	r, m	34
Acetylene-dicarboxic acid- dipyrrolidid	30—40 i. v.	r	15
Arecoline	20 i. p.	r, m.	28
Reserpine	10 i. v.	r, m.	63
Physostigmine	0,2 i. v.	r, m.	63
α -methyl dopa	100 i. p.	r, m.	58
Tetrabenizine	5—20 i. p.	c	12
Chlorpromazine	300 s. c. few days	c	30
p-methoxy-dopamine	100 i. p.	m	48
Chloreathylamine	75 i. p.	r	42
R S - 86	5 i. p.	m, r.	29

**TREMOROGENIC SUBSTANCES OF PARKINSONIC TYPE WHICH
INCREASE THE ACTIVITY OF OXOTREMORINE**

Oxotremorine + CuCl ₂	10 i. p.	r, m.	50
Oxotremorine + 5-HTP	100 i. p.	r, m.	50
Oxotremorine + CS ₂	50 i. p.	r, m.	54
Oxotremorine + Chlorpromazine	5 i. p.	r, m.	50
Oxotremorine + Natriumazid	3 i. p.	r, m.	54

Table IV

**TREMOROGENIC SUBSTANCES OF NON-PARKINSONIC TYPE
WHICH CAUSE REST TREMOR**

	Dosis mg/kg	Animals	References
Veratramine	10 i. p.	r, m.	46
LSD	1 i. p.	m	2
5-hydroxytryptophane	100—200 i. p.	m	7
Amino-triphenylpropane	30 i. p.	m	2
Nicotine	50 i. p.	rb	9
Cyclopium toxin	0,5 i. v.	r, m.	52
2-imino-3-(2', 6''-dichlorphenyl)- thiasolidine	10 i. p.	r, m.	3
Acrylamid	r 100 per os 2 x weaky c 40—50 i. v. 3—6 days	r, c.	23, 5, 32
Prenderol (2,2-diethyl, 1, 3-propa- nediol)	0,2 i. v.	r	6
Phencyclidine	10 i. p.	m	35
3, 4-dimethylphenethylamine (3, 4-DM)	40—60 i. p.	r	47
Ibogaine	10—20 s. c.	m	67
Adipiodone	2,0—2,5 i. v.	m	19
Harmine	15 i. p.	m	65
Harmiline	10—30 i. p.	r, m.	10
Hydralasine	40 i. p.	m	58
β, β' iminodipropionitrile	100 s. c. 3x	mo	23a

SUBSTANCES WHICH CAUSE NON-PARKINSONIC TYPE RIGOR

Ryanodine	0,150 i. p.	r	61
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Table III

SUBSTANCES WHICH CAUSE OF REST TREMOR AFTER APPLICATION INTO THE CNS

	Dosis mg/kg	Animals	References
Deferoxamine G. P.	10 γ	r	55
Carbachol G. P.	2—10 γ	r	43, 18, 16
3, 4-dimethoxyphenylethylamine St.	1—5 γ	r	33
5-HT N. N.	0,2—2 γ	r	24
5-HTP N. N.	0,2—2 γ	r	24
Mescaline St.	1—5 γ	r	33
α , α'' -dipyridyl N. N.	1—5 γ	r	53a
Zinc-acetate i. c.	1—16 γ	r, m.	8
Oxotremorine	2—4 γ /m	r	40
Continuase infusion of ACH in G. P.	1 γ /m	r	44

SUBSTANCES WHICH CAUSE PARKINSONIC TYPE RIGOR

Trifluoperazine	10 i. p.	r	43, 60
Fluphenasine	10 i. p.	r	40
Perfenazine	5 i. p.	r	36
Reserpine	Mo 0,25 per os 3 x r 7,5 i. p.	Mo, r.	40, 37, 49
Bulbocapnine	7 i. p.	r. m.	66, 40
2, 4-dichlorophenoxyacetic (2, 4-D)	100 i. p.	r	11
Derivatives methoxyphenylethyl- amine (so called Ernst's subst.)	—	r, m.	20
p-methoxidopamine	100 i. p.	m	48
Centaurea repens L.	Food in 10—20 days	horse	64
MnO ₂	200 s. c. 2 x in 40 days	Mo	38

Table V

**SUBSTANCES WHICH CAUSE INTENTIONAL WILSON TYPE TREMOR
WHEN GIVEN PARENTERALLY**

	Dosis mg/kg	Animals	References
Diethylcysteamine	50 s. c.	r, m.	59
CCl ₄	700 s. c.	m	56
Guanetidine	25—50 i. p.	m	17
Catapresan (rest tremor)	50 i. p.	m	31

**SUBSTANCES WHICH CAUSE PARKINSONIC TYPE RIGOR
WHEN GIVEN INTO CNS**

Carbachole (n. niger)	5—10 γ	r	43
Oxotremorine (n. niger)	5	r	43
/(4-(1-naphtylvinyl)-pyridine/HCl) (G. P.)	10 γ	r	53

**SUBSTANCES WHICH CAUSE INTENTIONAL TREMOR
OF NON-WILSON TYPE**

Thioacetamide	170 s. c.	r	56
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SPONTANEOUS TREMOR IN LABORITARY ANIMALS

The dog	1, 62
The cat	39
The mouse	45
The chicken	26

SUBSTANCES WHICH GIVE CHOREATIC-LIKE MOVEMENTS

2,2',2"-tripyridine	0,25 i. p.	r, m.	55,25
3-acetylpyridine	100 i. p.	r, m.	27

Mo = monkey, c = cat, rb = rabbit, d = dog, m = mouse, ch = chicken
 N. N. = nucleus niger, St = striatum, G. P. = globus pallidus, i. c. = intra
 cranial.

METODE ZA IZAZIVANJE BOLESTI EKSTRAPIRAMIDALNOG SISTEMA

KRATAK SADRŽAJ

Autor je pokazao da se mogu izazvati praktično sve tipične bolesti ekstrapiramidalnog sistema, kao što su Parkinsonova bolest, Wilsonova bolest, horeatički pokreti. Osim toga, opisane su i druge vrste tremora statičkog, ali ne parkinsonskog tipa, intencionog tremora, koji nije Wilsonovog tipa itd. Zatim su opisane metode izazivanja eksperimentalnog rigora. Nabrojane su i takve eksperimentalne manifestacije tremora koje reagiraju samo na inhibitore polisinaptičkih puteva ili samo na glicin. Skupljene su na jednom mjestu sve dosada poznate metode za izazivanje ekstrapiramidalnih oboljenja, i to na različitim životinjama, i upozoreno je koje od njih najviše se približuju po svojim biokemijskim osobinama sličnim bolestima u humanoj patologiji.

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DISCUSSION

Barbeau: Another explanation for guanethidine and catapressan action: it may be trough feedback information from peripheral nerve system. In the striatum we find a marked change in the turnover of dopamine to HVA, after small doses of guanethidine or catapressan. Thus, somehow, the striatum is informed of what is taking place at the periphery or even in the brain stem and reacts by modifying the dopamine content and thereafter changing the delicate balance between other important amines. This feedback control mechanism may be an important function of the striatum.

Stern: Thank you dr Barbeau for your interesting informations.

Cox: Please could you tell me more about the tremor produced by p-methoxidopamin with respect to 1) Time course of tremor, 2) Nature of tremor, 3) Is it antagonised by L-dopa, 4) Mode of action.

Stern: Tremor caused by methoxidopamin is a very good model because it simultaneously causes both tremor and rigor in mice and rats. I don't know if it can be antagonised by L-DOPA. The mechanism of acting is explained by Ernst theory.

Vizi: I wonder how can you explain the effect of guanethidine on inducing Wilson-type tremor. There is a great discussion in literature whether guanethidine is capable of passing the blood-brain barrier. It has been found that guanethidine reduces the NR-content of the brain (Pfeifer, Vizi), however, by others it was not confirmed. Do you not think, that the effect of guanethidine on adrenergic system may play some role in causing this type of tremor. And what about catapressan? How does it work?

Stern: Catapressan as well as guanethidin passes the haemoencephal-barrier in lave amounts. Thus the increasa of Cu occurs in brain and liver. It is probable that the small doses wich do not generate tremor do not pass the barrier niether. These sorts of tremors resemble very much to Wilson tremor, because thay can be impeded by penicilamin. It is interesting that catapressan produces the clinical picture of a rest-tremor and yet it can be inhibited by penicilamin and not by atropin.

Schnieden: I wonder if you would like to give your views on how carbon tetrachlorid produces tremor and why penicillamine inhibits form of tremor?

Stern: CCL₄ probably produces tremor by demaging liver and brain which leads to gathering of Cu. Penicillamin acts by linking Cu to itself.

Bernard: 1) How long after an experimental virus infection in the chicken does tremor manifest itself? 2) Have specific lesions been found in the chicken brain after this type of virus infection?

Stern: The infection of chicken with that virus causes strong tremor which lasts 3 or 4 days when chicken day. Mephenesin doesn't prolong the life of infected chicken.

Bruggencate: I would like to ask two questions to Prof. Stern:
1) As to the chicken-tremor you described, I would like to know what kind of virus has to be applied, and how it acts; e. g. does it cause degeneration in cerebellum or somewhere else? 2) Are the tremor types being not influenced by glycine?

Stern: 1) It is a virus which is applicated intracranially. I know neither mechanism of acting nor whether it damages cerebellum.

2) Many types do not react on glycine, but tremor developed by cyclopium toxin reacts well.

György: Do not you think that atropine methylbromide in high doses can penetrate the blood-brain barrier of the cat?

Stern: I agree with you.



W. OELSZNER, H.-D. FISCHER,
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CHOLINERGIC INDUCED TREMOR AND BRAIN ACETYLCHOLINE LEVELS

Tremor inducing doses of muscarine-like central cholinomimetics give rise to an increased brain acetylcholine content (13, 14, 21). On the other hand tremor antagonizing doses of atropinic agents prevent this increase and given alone decrease brain acetylcholine levels (10, 13, 14). From this finding the question arises, whether these cholinomimetics act by the increased amount of acetylcholine or by direct actions on muscarinic receptors (5, 6, 13).

Initially, we have investigated the correlation between arecoline induced tremor and brain acetylcholine levels in young chicks and rats (25, 26).

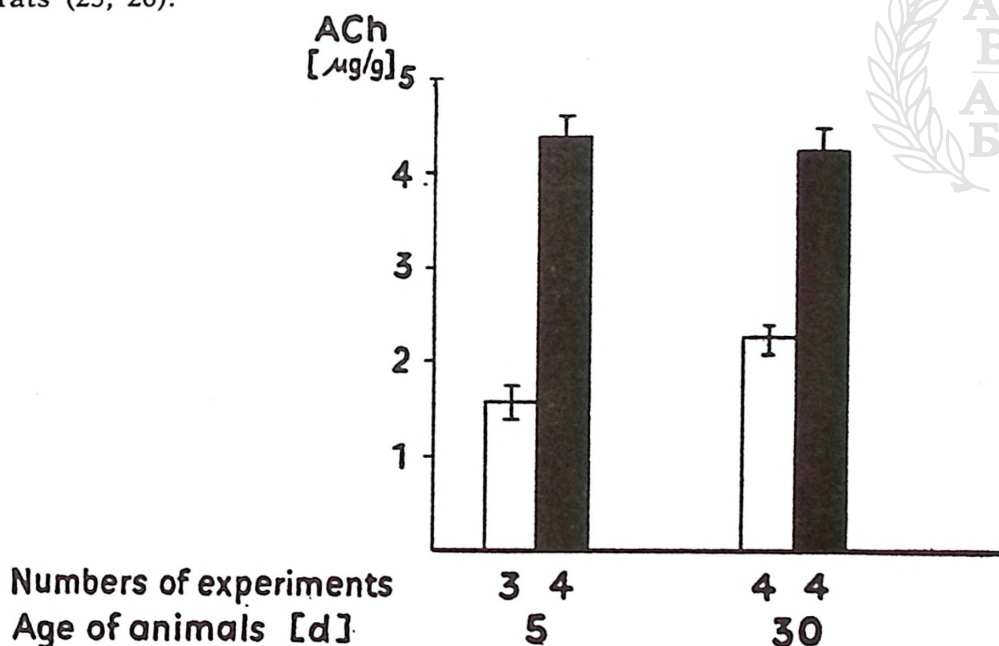


Fig. 1

Effect of arecoline on total brain acetylcholine (without cerebellum) of chicks aged 5 and 30 days.

Open columns: control animals

Dark columns: 5 min after 6 mg/kg arecoline i. p.

In chicks (Leghorn) aged 5 and 30 days arecoline in doses of 3 and 6 mg/kg i. p. produced a marked but short-lasting tremor for about 10 minutes. With oxotremorine in dosage levels up to 1 mg/kg the same symptoms only longer in duration could be obtained. In all experiments the intensity of tremor was related to dosage but differences due to age never were found. The effect of arecoline on total brain acetylcholine is demonstrated in Fig. 1. The low values of acetylcholine in chicks aged 5 days reached the same elevated amounts as in 30-days old animals. An effect of this magnitude, i. e. an increase by about 180%, has not been described in adult animals.

In rats (Wistar) aged 10 — 300 days 10 mg/kg arecoline i. p. induced a pronounced short lasting tremor without differences in intensity or duration due to age. When the same dose of arecoline was injected into 5-days old animals no tremor response could be obtained. The brain acetylcholine levels in control rats of different age and in rats pre-treated

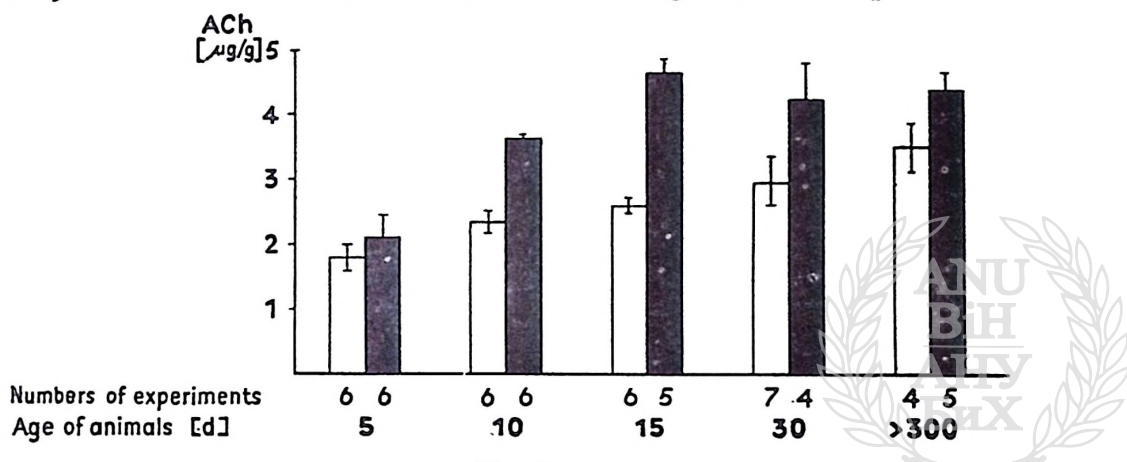


Fig. 2

Effect of arecoline on total brain acetylcholine (without cerebellum) of rats aged 5 — 300 days.

Open columns: control animals

Dark columns: 5 min after 10 mg/kg arecoline i. p.

with arecoline are presented in Fig. 2. On a wet weight basis there was a marked augmentation in the normal acetylcholine levels with increasing age. Arecoline produced a significant increase in the brain level of acetylcholine only in rats aged 10 days or more. In 5-days old animals there was a small increase of about 15%, but this effect was not significant.

Till now only few papers dealing with the different influences of cholinomimetics and cholinolytics on the acetylcholine content of various regions of rat brain are available (11, 18). For this study we used a dissection technique described by Popov et al. (24) and estimated the levels of total acetylcholine in eight areas of rat brain before and after injection of arecoline, RS-86, eserine and scopolamine (8). The normal amounts in certain regions varied considerably with lowest values in cortex and pons, higher levels in hippocampus, thalamus and hypotha-

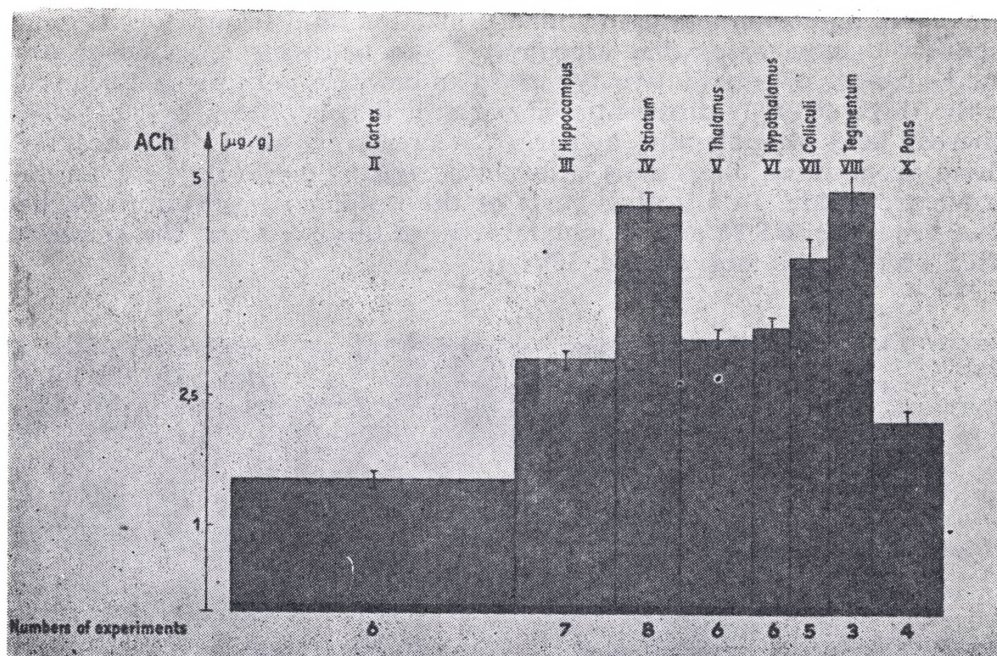


Fig. 3

Acetylcholine content of different regions of rat brain.

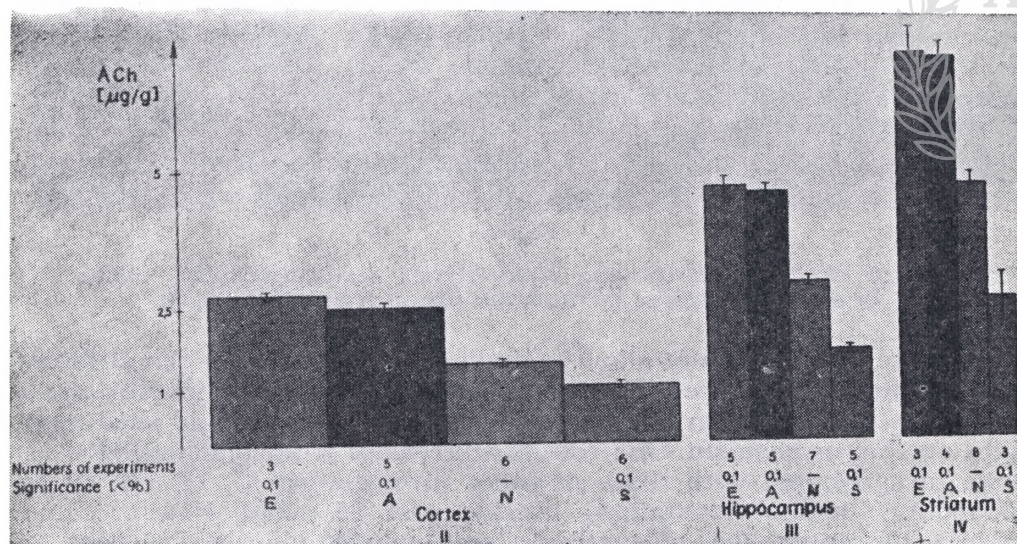


Fig. 4

Effect of cholinomimetics and cholinolytics on acetylcholine content of different regions of rat brain (telencephalon). Calculation of significance by Duncan's test.

N: control animals

E: 15 min after 0.5 mg/kg eserine i. p.

A: 9 min after 20 mg/kg arecoline i. p.

S: 30 min after 0.5 mg/kg scopolamine i. p.

lamus and highest amounts in colliculi, striatum and tegmentum (Fig. 3). Figure 4 demonstrates that arecoline, RS-86 and eserine in doses producing tremor of equal intensity caused an increase in brain acetylcholine in the three strongly differentiated parts of telencephalon by 50 — 80%, whereas after scopolamine a marked decrease by about 45% occurred. These pronounced alterations in brain acetylcholine levels were limited to telencephalon. In all other parts of the brain no significant changes after arecoline, RS-86 and scopolamine were observed and the elevation after eserine did not exceed 30% (Fig. 5).

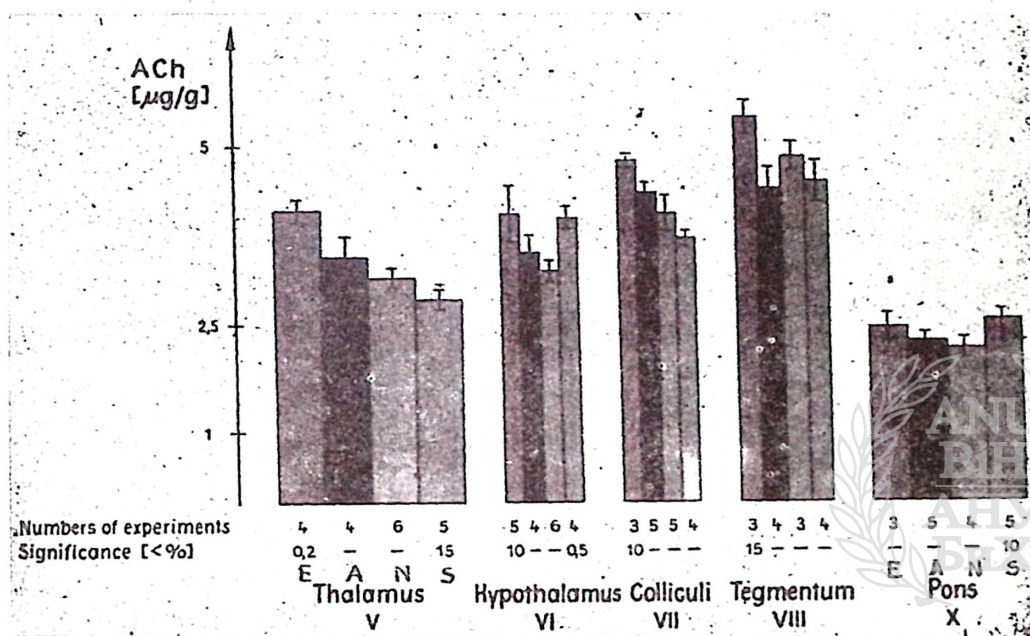


Fig. 5

Effect of cholinomimetics and cholinolytics on acetylcholine content of different regions of rat brain (brain stem). See Fig. 4.

These results did not allow to draw conclusions about the different steps of turnover of acetylcholine. It was suspected that in vitro experiments with slices from telencephalon and brain stem would shed more light on this mechanism. Our results with slices from telencephalon (7) were in agreement with those of other investigators (3, 13, 17). In an incubation medium containing eserine and 25 mmol KCl arecoline mainly promoted the biosynthesis and scopolamine the output of acetylcholine. In our experiments with slices from brain stem no significant changes could be obtained (Fig. 6).

In the presence of an anticholinesterase acetylcholine is released from the cerebral cortex and other parts of the brain (19, 22). Stimulation of afferent pathways or injections of atropinic agents increase the output. But till now only the acetylcholine release from higher parts of the brain, i. e. cerebral cortex, caudate nucleus and thalamus has

been investigated. We have repeated and confirmed these results mainly obtained with cats and rabbits using push pull technique in rats. The spontaneous release and also the evoked output after 1 mg/kg scopo-

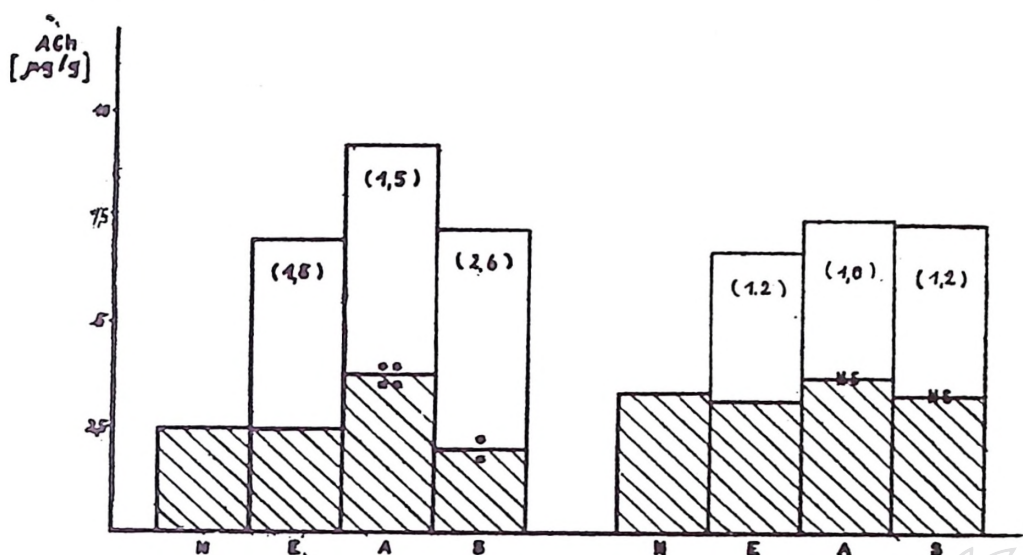


Fig. 6

Effect of arecoline and scopolamine on the content (shaded) and output (white) of acetylcholine (ACh) from slices of telencephalon (left) and brain stem (right) of rats during incubation.

N: tissue ACh-content before incubation.

E: ACh-content and released ACh after 120 min (incubation medium modified from Kalant and Grose, 25 mM KCl, 10^{-1} mM eserine).

A: like E in presence of 6×10^{-3} mM arecoline.

S: like E in presence of 2×10^{-3} mM scopolamine.

Calculation of significance by Student's t-test: $p < 0.01$, $p < 0.05$, NS = not significant. Ratio (medium-ACh: tissue-ACh) in parenthesis.

lamine from caudate nucleus and thalamus was very similar. The spontaneous release from substantia nigra however was much lower and the effect of scopolamine did not exceed 10% (Fig. 7). Oxotremorine also caused an increased release of acetylcholine from caudate nucleus, the output from substantia nigra was influenced only to a much lower degree.

From these findings and further results of other investigators it seemed possible to draw some conclusions about the open questions mentioned above. In our studies on chicks with arecoline and oxotremorine we could not confirm a fall-off of response with age described by Bowman and Osuie (4) using tremorine. There is some evidence that the developing resistance to tremorine in chicks but not in mammals depends on the existence of two different liver microsomal enzymes metabolizing tremorine to oxotremorine and oxotremorine to inactive metabolites respectively (16, 27). More important is our observation that in chicks and especially in rats there was a close correlation between

elevation of total brain acetylcholine and production of tremor. These data suggest that the cholinergic induced tremor may be related to the increased amounts and — according to our push pull experiments — also to an increased output of acetylcholine. But our further results do not support this assumption. In accordance with Cox and Potkonjak (5, 6) we believe that there is no causal relationship between these two effects and the inability of arecoline in increasing brain acetylcholine and in producing tremor in 5-days old rats seems to be related to the low stage of brain development.

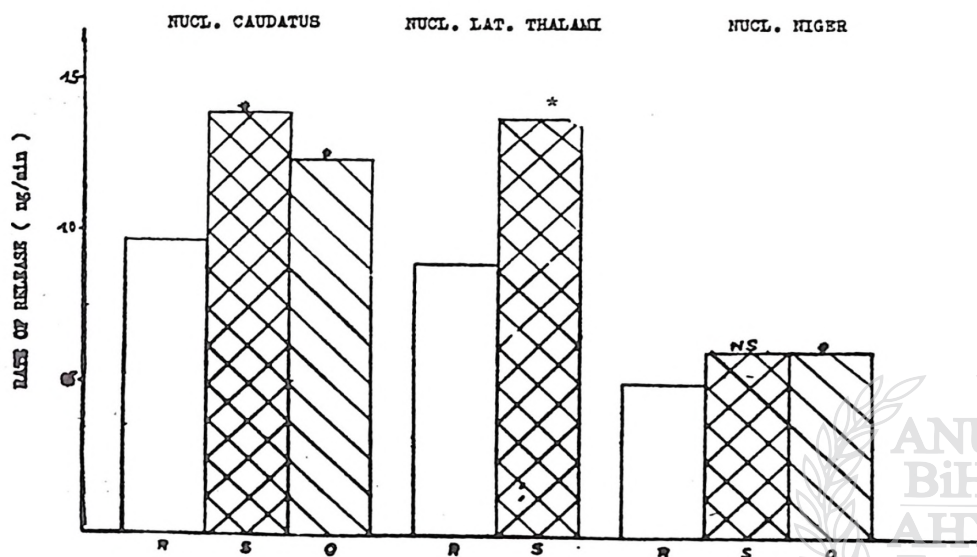


Fig. 7

Acetylcholine release from different regions of rat brain (1,25 g/kg urethane i.p.).

R = spontaneous release

S = scopolamine 1,0 mg/kg i. p.

O = oxotremorine 0,25 mg/kg i. p.

NS = not significant

= $p < 0,01$

In our experiments with intact animals pronounced differences in the reactions of various regions of the brain could be demonstrated. A significant rise or decrease in brain acetylcholine levels was limited to cortex, hippocampus and striatum, i. e. parts of telencephalon. These alterations in acetylcholine content induced by central cholinomimetics and cholinolytics were related to distinct changes in biosynthesis and depletion of acetylcholine as has been shown in our in vitro experiments confirming the results of other investigators. But in former studies only slices from cortex or homogenates of whole brain have been used and informations about the reactions of lower parts of the brain are not available (3, 23). In our present studies in slices from brain stem no significant alteration in turnover or content of acetylcholine could be detected and these results are in agreement with our findings in intact

animals. It is also possible to explain the obtained differences in the spontaneous and evoked release of acetylcholine from caudate nucleus and substantia nigra with these results in vivo and in vitro.

At present the mechanism by which cholinomimetics and cholinolytics influence the turnover and amount of brain acetylcholine is uncertain. The results of our in vitro studies provide good evidence of a similar mode of action of arecoline and high concentrations of potassium (7). In both cases the effects are restricted to telencephalon and closely connected with a generally increased brain metabolism. This rise in respiration and glycolysis shows marked differences in the different brain areas and is especially pronounced in telencephalon (12). These data suggest that cholinergic induced changes in brain acetylcholine levels and brain metabolism are causally related and differences in various brain areas can be explained on this basis.

As Cox and Potkonjak (5, 6) have already pointed out it does not seem likely that the tremorgenic actions of muscarine-like central cholinomimetics are related to the increase in brain acetylcholine levels. The finding in the present work confirms these conclusions especially taking into consideration some observations published by George, Haslett and Jenden (9). These authors have reported blocking of oxotremorine induced tremor in rats only with transections passing through the caudal midbrain or anterior pons. After transections in a higher level rostral to the red nucleus tremor remained unchanged. These data provide good evidence that the tremor originates in parts of the brain, in which we did not find significant changes in vivo and in vitro. The effects of cholinomimetics and cholinolytics are limited to telencephalon and from these results one may conclude that this area is not necessary for production of cholinergic induced tremor. These and further observations especially the corresponding peripheral and central muscarinic activity of tertiary cholinomimetics and cholinolytics (1, 2, 15, 20) provide good evidence that the tremorgenic actions of cholinomimetics and the tremor antagonizing actions of cholinolytics are related to a direct action on postsynaptic muscarinic receptors.

These investigations were supported by grant of the Ministry of Science and Technic of German Democratic Republic.

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TREMOR IZAZVAN HOLINERGICIMA I MOŽDANI ACETILHOLINSKI NIVOI

KRATAK SADRŽAJ

Poznato je da je tremor izazvan centralnim holinomimeticima praćen povišenjem totalnog acetilholina mozga sa istim vremenskim tokom. Mi smo istraživali vezu između ta dva efekta.

Kod pilića (Leghorn) starih 5 do 30 dana nismo mogli naći razlike u intenzitetu arekolinom izazvanog tremora koji ovisi o starosti. Kod mladih pilića niske vrijednosti moždanog acetilholina dostigle su iste uvećane količine kao i kod životinja starih 30 dana. Kod štakora starih

5 dana arekolin ne izaziva tremor, a primijećeno je samo malo i statički neznatno povećanje u nivou acetilholina. Kod životinja starih 10 i više dana tremor i znatno povećanje u acetilholinskom sadržaju bili su izazvani slučajno. Kod odraslih štakora povećanje u moždanom acetilholinu prouzrokovano arekolinom i RS-86 i povećanje nakon skopolamina bilo je ograničeno na telencefalon (cortex, hipocampus, striatum). U svim drugim moždanim zonama nisu primijećene uočljivije promjene. Iste razlike mogu se demonstrirati i u in vitro eksperimentima sa režnjevima iz telencefalona i moždanog stabla. Alternacija u biosintezi i deplecija acetilholina pomoću arekolina, skopolamina i visoke koncentracije kalijuma našle su se samo u režnjevima iz telencefalona. Količine acetilholina iz moždanog stabla ostale su nepromijenjene. Ovi rezultati se slažu sa eksperimentalnim proučavanjem spontanog i skopolaminom izazvanog oslobađanja acetilholina iz raznih dijelova mozga štakora, upotrijebivši puskpull-tehniku. Spontano oslobađanje je bilo mnogo manje iz zone substantia nigra i zone retikulata nego iz nucleus caudatus putamena, nucleus lateralis thalami ili cerebralnog cortexa. Skopolamin je izazvao pojačano oslobađanje acetilholina samo u višim dijelovima mozga. Efekt u supstanciji nigri nije prelazio 10%.

Ovi su rezultati razmotreni u odnosu na mogući način djelovanja tremorgeničkih holinomimetika.

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DISCUSSION

Schnieden: Could you give me some information about the drug RS 86.

Oelszner: RS-86 is a central cholinomimetic like arecoline and oxotremorine and possesses similar effects (increase of acetylcholine in brain tremor, inhibition of nociceptive reactions). It is produced by SANDOZ — Basel.

György: 1) How long lasts the effects of arecoline on the brain ACh-level? 2) Have you examined also the activity of OT on the amine level of the different parts of the brain?

Oelszner: Total brain acetylcholine in rats increased after ——— 25 mg/kg i. p. with maximum effect between 5 and 10 min after injection and then decreased (H. — D. Fischer and W. Oelszner, *Acta biol. med. germ.* 18, 443, 1967).

We have not used oxotremorine, but we believe this drug may act in a similar way.

Cox: Could you, please, tell me about the location of the increase of acetylcholine in brain slices by oxotremorine? Do you think that this acetylcholine is predominantly intracellular and not released into the extracellular fluid? In the acetylcholine coming out of damaged tissue in slices. It is interesting that the highest output of acetylcholine into the incubating medium comes in the scopolamine experiments this drug brain an anti-tremor agent.

Oelszner: We have investigated acetylcholine content in whole brain slices and with this method no conclusions about the location of the increase of acetylcholine are possible. Arecoline predominantly increases biosynthesis and the increased output into the medium may be an overflow, the increased output in vitro and in vivo has already been described by other authors.

Vizi: May I have your opinion on the role of actual content of ACh in producing tremor by oxotremorine or by arecoline. Tremorgenic agents with muscarinic activity increase the ACh content of brain (Holmstedt and Lundgren, Ann. N. Y. Acad. Sciences 142, 126—142, 1967; Oelszner et al. Acta medica Germanica; Cox and Potkonjak, Brit. J. Pharmacol. 35, 295—303, 1967, ect) but did not increase the acetylcholin release from the nerve terminals of Auerbach plexus and the cortex of the rat (See: Vizi and Knoll, this Symposium). Dount you think that these fact favour the direct effect of oxotremorine resulting in tremor? Which is the crucial point in tremor: the release of ACh or the content? Oxotremorine mimicks the effect of ACh on muscarinic receptor and does not release it.

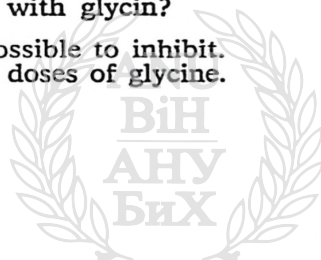
Oelszner: We believe that neither the increased acetylcholine content nor an increased output of acetylcholine in the brain after central cholinomimetics is responsible for cholinergic induced tremor. These agents act directly on postsynaptic muscarinic receptors and on this basis induce tremor or other central effects.

Župančič: Doctor Oelszner, you found significant differences in acetylcholine level, e. g., in the corpus striatum after administration of arecoline. One can imagine that these differences might be even greater, if breacing down your area wich you determined acetylcholine, say in nucleus caudatus instaed of in the whole striatum.

Oelszner: It is nearly impossible to dissect the little rat brain to a further degree because this needs time in which acetylcholine is destroyed by acetylcholinesterase.

Štern: Is it possible to inhibit other kind of tremor with glycin?

Oelszner: We have some observations that it is also possible to inhibit. Arecoline or oxotremorine induced tremor in rats with high doses of glycine.



TUBOCURARINE INDUCED SHIVERING IN CATS: INFLUENCE OF SPINAL CORD LESIONS AND ANTIPARKINSONIAN DRUGS

1. SPINAL MOTOR SYSTEMS IN CURARE SHIVERING

FELDBERG and coworkers (7) first described a tremor state in cats resembling cold shivering, evoked by perfusion of the brain's third ventricle with a tubocurarine solution. Considering the divergent opinions existing on the mechanism of tremor, it seemed worth while to investigate this type of tremor with neurophysiological techniques. The first part of the analysis was carried out some years ago in collaboration with H. D. Henatsch (3, 4, 11, 12). For a better understanding of recent results the main changes in spinal motor systems accompanying curare shivering (CS) will be summarized briefly.

Fig. 1 demonstrates activity in α - and γ -motor systems during CS. A—C show the periodically occurring activity of motor units and muscle spindles during CS. In A and B (triceps surae unstretched) both Ia and II afferents are activated during tremor periods. On the other hand, in C (triceps surae stretched for 1 mm and 10 mm, resp.) the spindle discharges decrease in tremor bursts. In this state, obviously the shortening of motor fibres during the tremor period mainly determines the spindle discharges. Taken together, A and C suggest that the activity of both γ - and α -motoneurons is enhanced during periods of shivering. Coactivation of α - and γ -motor systems under curare is further illustrated in D—F. D shows stretch activated discharges of a single α -motoneurone superimposed on background impulses of γ -motoneurons in the control situation. In E, under curare perfusion, the stretch reflex discharge of α -motoneurons increased. In F, still under curare perfusion, flaxedil was injected to interrupt the γ -spindle loop. Stretch induced activity in α -motoneurons now is less than in E, but still higher than in D. The difference between recordings F and D is attributable to increased α -drive, the difference between E and F to increased γ -drive.

There are indications of a predominant spinal origin of the tremor oscillations in CS (11, 12). On the other hand, there seems to be no

pacemaker-like activity in γ -motoneurons, e. g. no γ -discharge locked to tremor oscillations (3, 12). A way out of this conflict could be a change in receptor sensitivity of muscle spindles induced via the dynamic or static part of the γ -system.

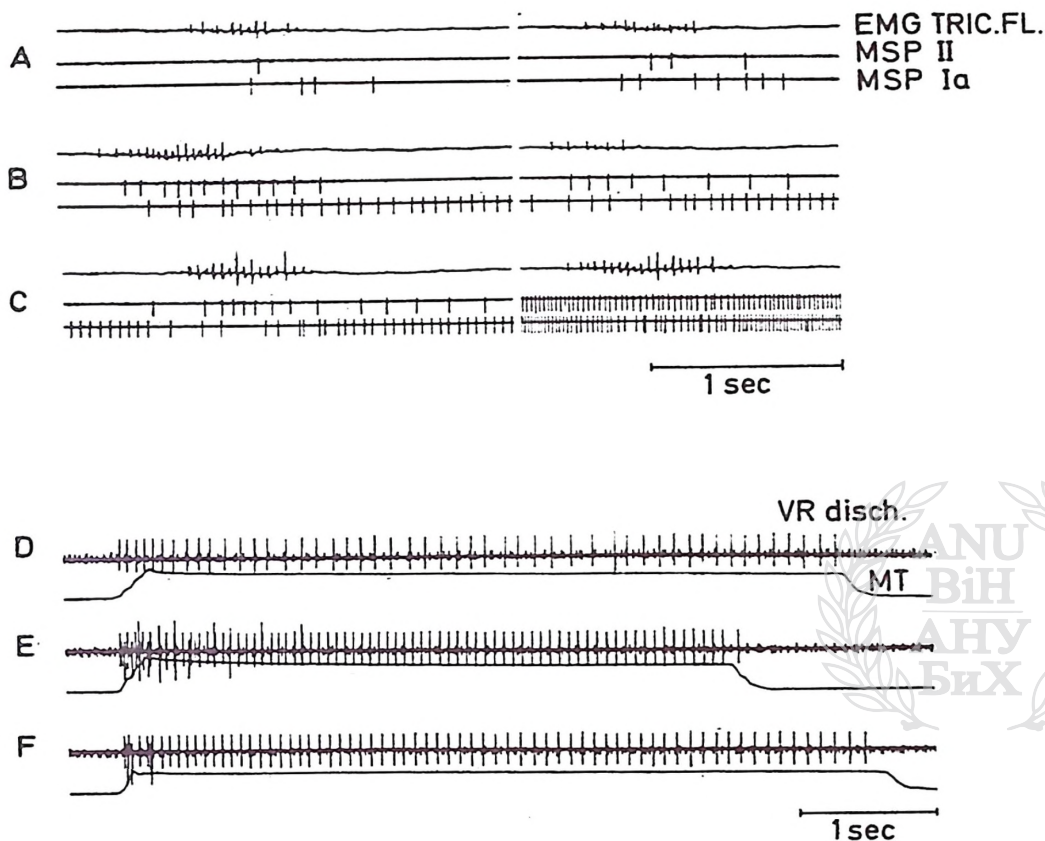


Fig. 1

Coactivation of α - and γ -systems during CS.

A—C: Records of EMG in forelimb extensor, and discharges of secondary and primary muscle spindle endings from triceps surae. Specimen taken during tremor periods and successively after the end of a previous stretch of triceps surae, with slack muscle (A, B) and stretched muscle (C, 1 mm on the left, 10 mm on the right).

D—F: Discharges of α - and γ -motoneurons recorded from a small ventral root filament (roots otherwise intact). D: Activation of a single α -motoneurone on muscle stretch before curare perfusion. E: Activation of several α -units during curare. F: Activity after interruption of the γ -loop (Flaxedil 20 mg/kg).

Lower trace: muscle tension (MT) (length of muscle extension identical in D, E, and F).

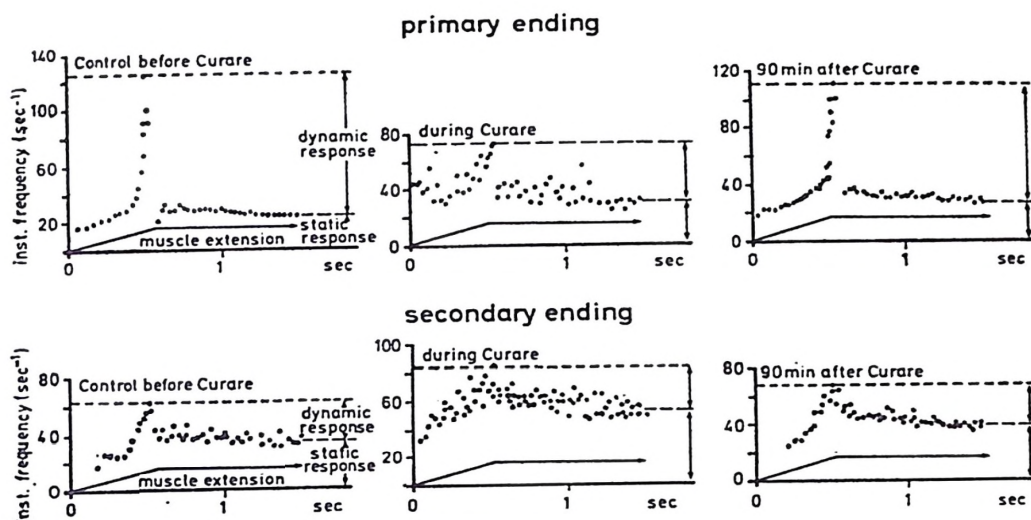


Fig. 2

Decrease of dynamic, and increase of static responses of muscle spindle endings during CS.

Plots of instantaneous frequency versus time for identical muscle extensions before, during, and after curare perfusion. Each dot represents a spike of a primary and secondary ending, respectively. The increase of the static response during curare is paralleled by an increase in the variability of discharge intervals (scatter of dots). (After 2).

Fig. 2 shows the discharge patterns of a primary and a secondary ending simultaneously recorded before, during, and after perfusion. The insets show the afferents' discharge rate during muscle extension (indicated below). Each dot represents an action potential, its distance from the zeroaxis indicating the instantaneous discharge frequency. Obviously, the dynamic sensitivity of the primary ending decreases considerably whilst the static discharge rate during extension increases, particularly in case of the secondary ending. In terms of feedback control in the stretch reflex arch, decreased dynamic sensitivity and increased static sensitivity should lead to instability of the system (c. f. 16).

Thus, in our opinion curare perfusion evokes a steady, primarily arrhythmic, descending drive to spinal motoneurones. The influence impressed upon the fusimotor system leads to changes in receptor sensitivity which should at least contribute to the development of tremor oscillations at a spinal level.

2. EFFECTS OF SPINAL CORD LESIONS ON SPINAL MOTOR SYSTEMS DURING CS

It was our general experience that small circumscribed lesions, differently located in the spinal cord, did not entirely abolish CS below the level of the lesion in question. However, there was always a diminution of CS in varying degrees. The relatively clearest effects were

observed with rather superficial lesions of the dorsolateral funiculus (DLF) which sometimes reduced shivering on the side contralateral to the lesions also.

Recording from ventral root filaments disclosed that shivering reduction caused by lesions in the DLF usually was accompanied by a reduction in the discharges of α -units. Fig. 3 shows an experiment with

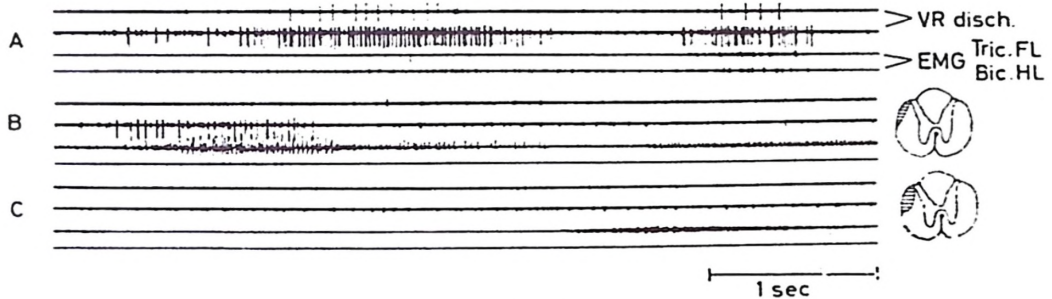


Fig. 3

Decrease of curare-induced discharges of α -units by lesions in the DLF. A: Background discharges of γ -units (small) and periodically occurring α -discharges during CS. EMG from forelimb and hindlimb muscles in the lower beams. B and C: Reduction of α -discharges and tremor in the hindlimb after lesions in the ipsilateral DLF, as indicated on the right. No significant change in γ -discharges. Increase of shivering EMG-discharges in the forelimb.

marked effects on CS. In A, during curare perfusion, shivering occurred periodically in fore- and hindlimb muscles, simultaneously with α - and γ -discharges. A superficial lesion in DLF (B) dramatically reduces electromyographic activity in the hindlimb muscle, parallel to a corresponding effect on discharges of α -units, while γ -impulses seem rather unchanged. The effects are somewhat accentuated in C with an enlarged lesion. Typically, after the lesion shivering intensity in the forelimb increased.

Discharge rates of γ -units, enhanced during curare perfusion were »normalized« only after hemisection of the cord. Recording from muscle spindle afferents gave results confirming this notion. Fig. 4 shows the response of a primary ending to muscle extension. During CS the dynamic response is reduced, the static enhanced (4B, cf. fig. 2), the discharges being more scattered than in the control. A lesion of the DLF only temporarily reduces the curare effects (C) the behavior of the receptor in D (9 min after lesion) being rather similar to B. A discharge pattern like the one in the control situation results only after extension of the lesion to include most of the VLF (E, F).

3. EFFECTS OF ANTIPARKINSON DRUGS.

CS seems a useful model to study changes of spinal motor functions occurring in a tremor-like state. However, the question remains, as to what extent this model is similar to clinically relevant tremor states. One point, which seemed worth being tested, is the effect of drugs used clinically to reduce parkinsonian tremor. Furthermore, we wanted to know if any eventual effect was due to a selective mechanism

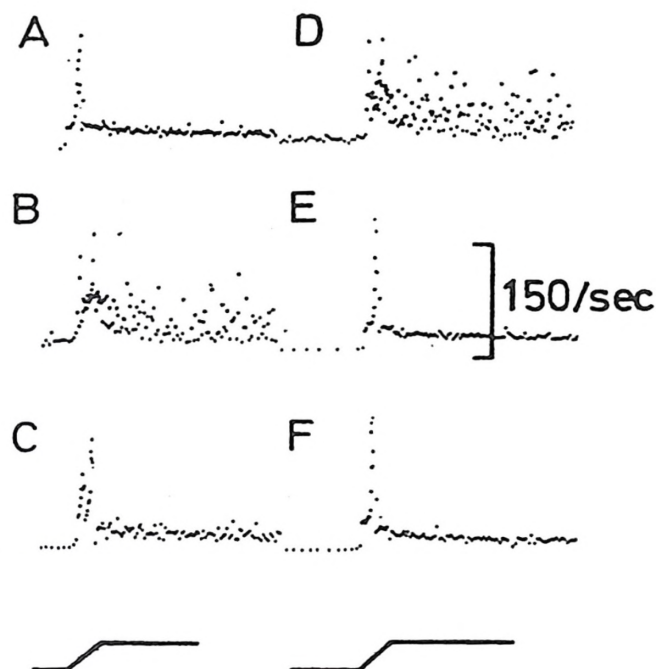


Fig. 4

Reversal of curare-induced changes in muscle spindle sensitivity by spinal cord lesions.

Plots of instantaneous frequencies of a primary ending versus time for identical muscle extensions, as in fig. 2. A: Control. B: Curare-induced decrease of dynamic and increase of static sensitivity. C: 1 min, D: 9 min after a DLF lesion as in fig. 3C. E, F: 1 min and 10 min after an additional lesion in VLF.

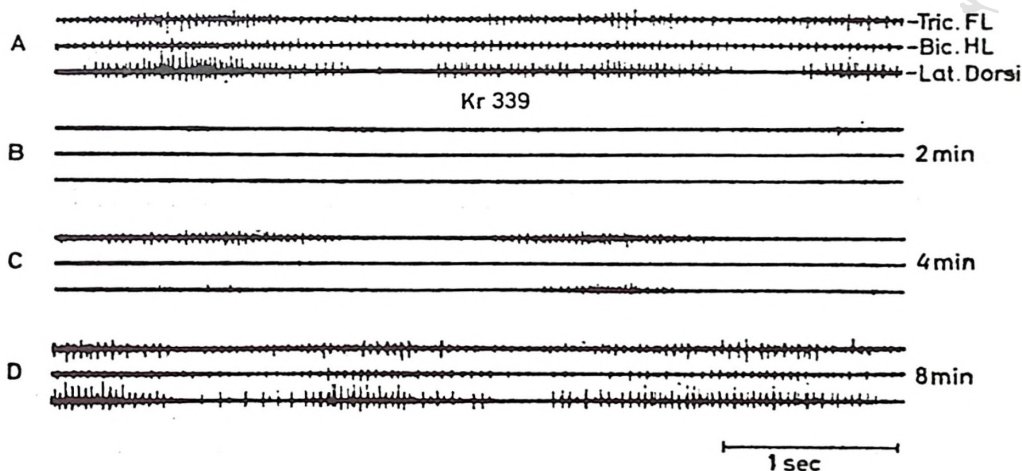


Fig. 5.

EMG records of curare-induced shivering, and its reduction by intravenous injection of an antitremor drug.

Recordings as indicated from forelimb triceps, hindlimb biceps and latissimus dorsi. (After 20).

or rather to an unspecific, general action. Fig. 5 illustrates the effect of Kr 339* on electromyographically recorded CS. Shivering was abolished by 1,6 mg/kg kr 339 for about 2 min and definitely reduced for 7 min. The behaviour of α - and γ -motoneurones was also investigated.

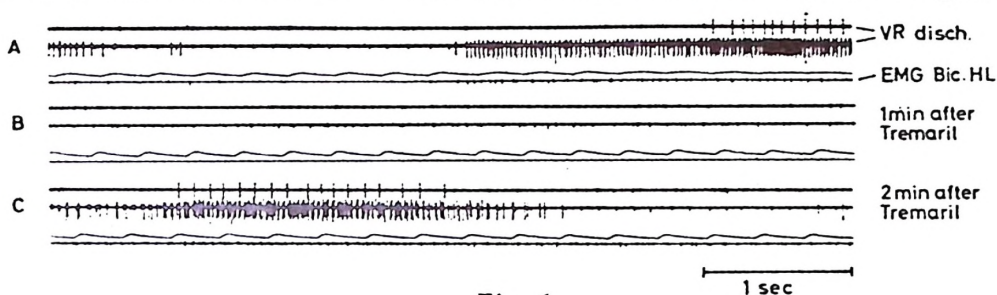


Fig. 6

Temporary reduction of curare-induced periodical α -discharges by an antitremor drug. (After 20).

Fig. 6 shows a reduction of α motoneurone discharges after a dose of tremaril* which abolished the shivering.

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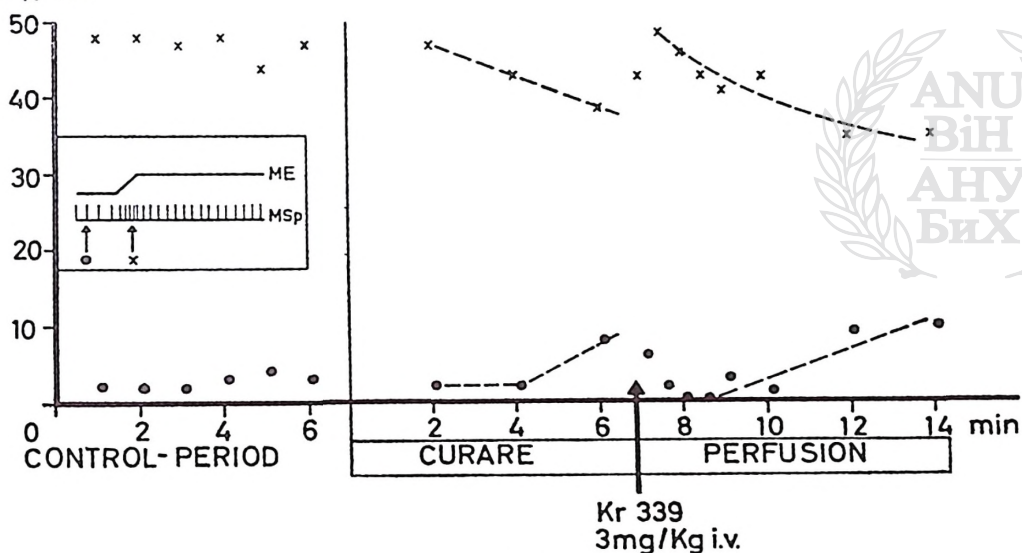


Fig. 7

Curare-induced changes in muscle spindle sensitivity, and the effect of an antitremor drug.

Plots of dynamic peak frequency (crosses) and spontaneous discharges (points) as indicated. Reduction of dynamics, increase of static sensitivity during curare. Temporary reversal of these effects by injection of antitremor drug.

* 2-phenyl-biciclo-(2, 2, 1)-heptane-2-carbonic acid (γ -diethyl-aminopropyl)-ester, Knoll AG, Ludwigshafen/Rhein.

* 9-(N-methyl-3-piperidyl)-methyl-thioxanthen-hydrochloride, Wander GmbH, Frankfurt.

On the other hand, discharges of γ -motoneurons definitely are not reduced. In fact, temporary increases were seen in several experiments shortly after injection of the drug. If there was a reduction of γ -impulses (always less clear than the effect on impulses of α -motoneurons), it occurred at a later time after injection.

Fig. 7 graphically demonstrates the behavior of 1) spontaneous discharges (muscle unstretched) and 2) dynamic peak frequency of a primary muscle spindle ending. Curare perfusion lead to a decrease of dynamic peak frequency and an increase of the tonic background discharges. Injection of the antitremor drug temporarily reversed the curare-induced changes in muscle spindle impulse pattern.

4. DISCUSSION

Tubocurarine applied to the surroundings of the third ventricle leads to a shivering tremor which obviously is accompanied by a complex change in reactivity of spinal motor systems. Taking into consideration the striking similarity with cold shivering, it is of interest that Birzis and Hemingway (1) were able to record in descending »shivering pathways« only arrhythmic discharges, asynchronous with the shivering ascillations. We also failed so deffect a clear synchronization of discharges of α -motoneurons in paralyzed animals, whilst γ -discharges never showed any synchronization even in unparalyzed cats. Our conclusion is, therefore, that in CS the shivering rhythm originates at a spinal level, and that in CS proprioceptive contribution plays a dominating role. There is evidence which suggests that the first conclusion is also valid for cold shivering (8, 19). However, the importance of the proprioceptive contribution compared to the interneuronal contribution is probably less in cold shivering (8, 20), as well as in animals with locally cooled spinal cord (17).

With local cooling of the spinal cord, shivering can be produced even in chronically deafferented animals (17). This suggests that spinal interneurons, perhaps, together with changes of the motoneuronal membrane properties (18), are correlates of tremorgenesis in this case. However, as to the spinal mechanisms responsible for the origin of shivering, the experimental approach with local cooling within the vertebral channel seems hardly comparable to a pharmacological model acting on centrencephalic structures.

Our results with limited lesions within the spinal cord agree with the previous notion that there is a rather diffuse, general descending influence, acting on α - and γ -motor systems and even on presynaptic inhibition (5). Motor activity may depend on coactivation of α - and γ -motoneurons (α - γ -linkage, 9, 10); reduction of shivering amplitude by spinal cord lesions with a variety of localizations would then be understood as a threshold effect, reducing the final input to α -motoneurons (either running directly and/or via the γ -loop) below firing level.

The finding, that superficial lesions within the DLF often had a relatively great effect, has yet to be explained. Since neither acute

pyramidotomy nor chronic lesions of the red nuclei abolish CS (ten Bruggencate and Weller, in preparation), a contribution of reticulospinal systems or of ascending paths must be considered.

Our experiments with injection of antiparkinsonian drugs show that these drugs are to some extent also effective in CS, »normalizing« all motor symptoms induced by curare perfusion. Concerning the effect on α - and γ -discharges, the reduction of activity of α - and γ -units showed the reversed order to the activity increase during curare perfusion: curare tends to activate γ -motoneurons earlier than α -units; whereas antitremor drugs depress α -units first, high amplitude units more strongly and earlier than low amplitude α -units or even γ -units. This fact suggests that both curare and antitremor drugs influence spinal motoneurons in a rather unspecific manner, seemingly largely dependent on cell size and, therefore, probably on general properties of cell membranes (6, 13). Again, this supports the view that curare sets free an elementary system which activates mechanisms inherent to the spinal cord. Biologically, the ideas expressed by v. Holst (quoted in 14) and Jung (14) still hold, i. e. that shivering tremor might be the sign of phylogenetically old mechanisms related to flapper movements.

G. TEN BRUGGENCATE

EFEKTI LEZIJA KIČMENOG STUBA I ANTIPARKINSONSKIH LIJEKOVA NA TREMOR MAČAKA IZAZVAN KURAREOM

KRATAK SADRŽAJ

Već ranije je analiziran tremor izazvan tubokurarinskom perfuzijom moždanih ventrikula s obzirom na kičmene alfa i gama motorne sisteme.

Glavni rezultati su bili: a) povećanje rasterećenja fuzimotornih neurona tokom kurare perfuzije, bez ikakvog znaka uspostavljanja ritmičke aktivnosti; b) ritmička rasterećenja alfa-jedinica, uništenih interupcijom vretenastih spojnika, koja indiciraju da ritam tremora potiče iz nivoa kičme; c) promjene osjetljivosti vretenastih završetaka mišića koje mogu biti važne za prethodne zaključke.

Izneseni eksperimenti opisuju efekte različito lociranih lezija kičmenog stuba na ponašanje alfa i gama motornih sistema u toku kurare tremora (KT). Da se unište svi neurofiziološki simptomi koji prate KT, bile su potrebne velike lezije. Ova činjenica potkrepljuje ideju da silazne aktivnosti u toku kurare tremora olakšavaju kičmene motoneurone na nespecifičan način i da se one ne prenose samo preko dobro uhođenih (određenih) ekstrapiramidalnih puteva.

Sasvim drugo pitanje se postavlja: da li KT može da bude model za istraživanje kliničkih supstanci koje su primijenjene kao antiparkin-

sonski lijekovi? Pokazano je da ovi lijekovi »normalizuju« neurofiziološke KT-simptome, premda je poznato da se moraju primjenjivati visoke doze. Čini se da KT može da bude upotrijebljen kao eksperimentalni model u neurofarmakološkim ispitivanjima.

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DISCUSSION

Stern: Have you tried to act upon tremor which you have developed in cat by atropin and methyl-atropin? In that way we could investigate antiparkinsonics. If this tremor can be inhibited by methyl-atropin, it means that it is of peripheral nature.

Bruggencate: I am sorry, we didn't test atropine or methylatropine. Thank you so much for your valuable suggestion.

Schnieden: Did you monitor the temperature of your preparation and did the antiparkinsonian agents, effective in modifying E. M. G. picture, also affect temperature of your preparations.

Bruggencate: We measured rectal temperature and kept it constant by a heating device. However, we have not done recordings of temperature and did not try to correlate it with curare perfusion. We did not pay attention to short time temperature drops, eventually related to injection of antitremor drugs. Therefore, any effect of these drugs on rectal temperature has probably escaped our observation.

L. GYÖRGY, B. GELLÉN, M. DÓDA, A. K. PFEIFER AND A. BITE

THE INFLUENCE OF CNS-DRUGS ON THE ACUTE TOLERANCE TO THE DIFFERENT ACTIVITIES OF OXOTREMORINE

Tolerance is known to develop to certain effects of chronically administered oxotremorine (OT) (1, 2). Acute tolerance to the tremorigenic action of OT has been described in cats decorticated (3), or given the drug into specific cerebral areas (4). In work done last year (5), we found that in mice premedicated on one occasion with tremorine or on two occasions with OT, some effects of OT — such as tremor, motor disturbances, etc. — weakened precipitately, within from a few to 24 hours. The activity of three consecutive intraperitoneal OT injections grew gradually less, as observed on the rotarod (6) (Table I).

Table I

ROTAROD ACTIVITY OF 3 SUBSEQUENT OT INJECTIONS
(GIVEN AT 0, AT 60 OR 90 MIN, AND AT 120 OR 150 MIN)

Dose and route	% mice n	mice dropped off the 1st	rotarod after the 2nd	3rd injection
0.5 mg/kg all i. p.	56	89	29	5
1.0 mg/kg all i. p.	40	100	50	25
0.5 mg/kg i. p., i. p., i. v.,	16	100	19	13
1.0 mg/kg i. p., i. p., i. v.	16	100	40	13

This decline cannot be ascribed to impaired absorption, because when administered intravenously the effect of the third injection was also very weak. We, further, demonstrated that repeated OT injections reduced nicotine and physostigmine lethality in the mouse.

In more recent work, we wished to find out whether acute tolerance might not develop to other effects of OT, and if so, by what mechanism.

We studied OT for its analgesic activity in randomized mice by the hot-plate method. 0.1 mg/kg of OT prolonged the reaction time in the control animals, but exerted no effect whatsoever on mice pretreated 24 hours earlier with 20 mg/kg of tremorine (Table II).

Table II

ANALGESIC ACTIVITY OF OT ASSESSED ON THE HOT PLATE

Pretreatment — 24 hours	n	Dose of OT, i. p. O hour	Increments in reaction time (seconds) (median and range)
NaCl	12	0.05 mg/kg	5.5 (—7 and 41)
Tremorine			
20 mg/kg	12	0.05 mg/kg	—1.5 (—13 and 9)
NaCl	12	0.10 mg/kg	32.5 (20 and 47) x
Tremorine			
20 mg/kg	12	0.10 mg/kg	0 (—24 and 22) xx

x — xx : $p < 0.01$

Tremorine administered twice daily for three consecutive days did not diminish the ability of OT to potentiate pentobarbital narcosis.

Spontaneous motility in mice was measured with the motimeter devised by Knoll and Vajnovszky (7) in 10 times 50 cm channels, in which the animals close the circuit each time they step over from one square into another. 0.05 mg/kg of OT substantially weakened spontaneous motility, even when administered repeatedly. In animals treated at five 90-min intervals with physiological saline, motility — as measured after each injection for 30 min — was found to decline substantially from one measurement to the other, presumably due to gradual extinction

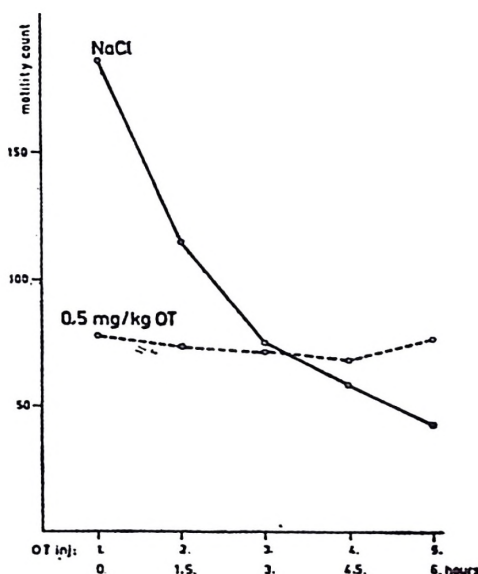


Fig. 1

of the orientatory searching reflex. (Fig. 1). In mice treated with 0.5 mg/kg of OT in the same manner, the first injection decreased motility to 41% of control value, but the subsequent three injections did not cause it to decline parallel with that value; on the contrary, the fifth increased it as compared with the saline-group.

In other experiments we found that premedication with OT did not inhibit the lethality of carbachol and neostigmine not passing through the blood-brain barrier; in contrast to nicotine and physostigmine lethality which repeated OT injections did reduce. On this basis we assumed that acute tolerance is of central nervous system origin and studied the question whether it is influenced by certain pharmacological agents that act on the CNS. Pretreatment with 40 mg/kg of phenobarbital prolonged the effect of OT and inhibits the development of acute tolerance. (Fig. 2).

% mice dropped off the rotarod after 0.5 mg/kg OT.

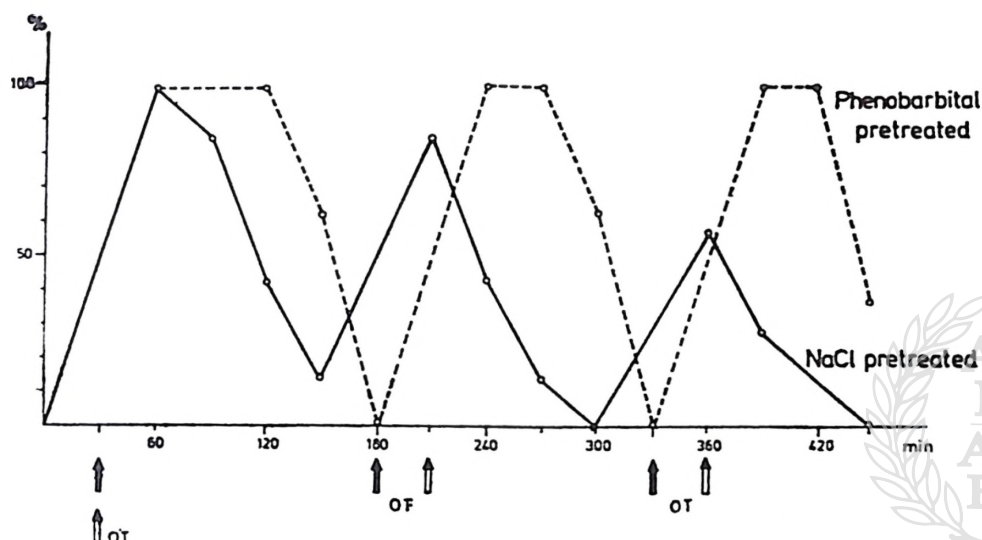


Fig. 2

When acute OT tolerance had already developed, the fourth injection of the drug was ineffective, but 0.05 to 0.1 mg/kg of haloperidol, which possesses anticholinergic activity, given before the fourth injection, re-established its effect (Table III). For that matter, these haloperidol doses failed to potentiate the activity of small doses of OT.

Table III
INFLUENCE OF HALOPERIDOL ON THE ACUTE OT-TOLERANCE
ASSESSED ON THE ROTAROD

Treatment after the 3rd 0.5 mg/kg OT-dose i. p.	n	Number of mice dropped off the rotarod after the 4th dose of OT	
NaCl	46	6	(13%)
0.05 mg/kg haloperidol	46	26	(57%)
0.10 mg/kg haloperidol	37	30	(81%)

Acute tolerance to OT was inhibited by 1 mg/kg of reserpine administered 18 hours before the drug; 10 mg/kg of tranlylcypromine, 2 mg/kg of desmethylinipramine or 2 mg/kg of DL-amphetamine accelerated it. Such small doses of the three last mentioned compounds possess no anti-OT activities.

The behavioral effects of, and vegetative reactions to, OT were studied on cats. When preceded by 0.5 mg/kg of atropine methylbromide, the same dose of OT elicited a typical rage reaction. Three or four daily OT injections repeated on three to four consecutive days — with atropine methylbromide given once daily — failed to slacken the intensity of the reaction, and no tolerance to it developed. Rage reaction was invariably accompanied by a sympathetic excitatory process. This was evident from our finding that in cats superficially anaesthetized with urethane, immobilized with gallamine, and premedicated with atropine methylbromide, the drugs, OT, arecoline, and pilocarpine caused contraction of the innervated, but not of the decentralized, nictitating membrane. This action is definitely central in origin, for ganglionic blocking agents and small doses of haloperidol or reserpine inhibit it. Its cholinergic origin is borne out by the fact that physostigmine increases it. This sympathetic response presents itself in animals pretreated with OT, and repeated injections of the drug heighten rather than lessen it.

To sum up, in the mouse tolerance develops to various effects of OT and is probably of central origin, possibly mediated by catecholamines. No acute tolerance to the stimulating effects of oxotremorine has been observed in cat-experiments.

L. GYÖRGY, B. GELLÉN, M. DÓDA, A. K. PFEIFER I A. BITE

UTJECAJ CNS-LIJEKOVA NA AKUTNU TOLERANCIJU RAZLIČITIH AKTIVNOSTI OKSOTREMORINA

KRATAK SADRŽAJ

Dvije ili tri konsektivno 0,5 do 1,0 mg/kg davane doze oksotremorina u mišu postepeno oslabe aktivnosti grupa za izazivanje koordinativnog motornog poremećaja (rotarod) koji izaziva tremor i pri reduciranju spontanog motiliteta. Jedna doza tremorina sprečava ova djelovanja oksotremorina, kao i njegovu analgetsku aktivnost, ali ne utiče na njegovu sposobnost jačanja pentobarbitalne narkoze. Dvije slijedeće doze 1,0 mg/kg oksotremorina reduciraju smrtonosnu toksičnost fizostigmina i nikotina, ali ne utiču na karbapol i neostigmin. Premedikacija fenobarbitonom sprečava razvoj koordinativne motorne smetnje izazvane oksotremorinom, a naredno liječenje vrlo niskom dozom haloperidola dovodi do intolerancije.

Nagoviješteno je da u razvoju akutne tolerancije na oksotremorin glavnu ulogu igra centralno proturegulatorno stimulativno djelovanje pokreta izazvanog aktivnošću grupa. Moguće je da je stimulativno djelovanje

stvo oksotremorina skriveno iza nekog drugog jačeg dještva. U eksperimentima sa mačkama nije ispitivana akutna tolerancija na karakteristični stimulativni efekt.

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DISCUSSION

Stern: I think that the tolerance to oxotremorine can be explained by acting of increased quantities of ACh upon cholinacetylase. It is well known that oxotremorine activates cholinacetylase, because of that more ACh is produces and that inhibits this ferment.

György: We have not examined the effect of oxotremorine on the cholinacetylase; we have some negative results about the role of acetylcholinesterase inhibiting activity of OT. We do not suppose that these activities of OT play a role in the tolerance phenomenon.

B. BOŠKOVIĆ, N. ROSIĆ AND V. VOJVODIĆ

**SOME PHARMACOLOGICAL ACTIONS OF OXIMES AND
CHOLINOLYTICS ON THE CARDIOVASCULAR EFFECTS
PRODUCED BY OXOTREMORINE IN ANAESTHETIZED RATS.**

The changes in arterial blood pressure are commonly seen after the administration of adequate doses of cholinergic drugs (anticholinesterase inhibitors, arecoline, pilocarpine, muscarine etc.) to most animal species (5, 6, 9). The rat appears to behave somewhat differently from other species. Anticholinesterase (anti-ChE) agents and other cholinomimetics cause a rise in blood pressure in rat, which is probably due to a direct action on the vasomotor center (4, 11, 14, 15).

Since the central actions of oxotremorine (OTR) may be blocked by atropine and related agents, it was reasonable to suppose that these are the manifestations of OTR muscarinic activity (3, 7, 8).

On the other hand, there are evidences that oximes could abolish the hypertensive effect in rats poisoned by anti-ChE compounds. This effect is probably due to direct anticholinergic actions of oximes, since it appears in animals poisoned by reactivatable and nonreactivatable organophosphorus compounds (12, 16).

Therefore, it was interesting to study the effects of oximes in cardiovascular changes induced by OTR. OTR was used instead of anti-ChE compounds in order to avoid the other effects these compounds may have, unrelated to their ability to inhibit cholinesterase.

MATERIAL AND METHODS

Male rats weighing between 200 and 250 grams were used. The animals were anaesthetized with urethane (0.7 ml of 25% urethane/100 gr., subcutaneously) and the arterial blood pressure was registered from the left carotid artery, using a mercury manometer (2). To avoid clotting,

heparin 500 i. u./100 gr., was given intravenously. A T-shaped cannula was inserted into the trachea and one arm of the cannula was connected by a rubber tube to a Mare's tambur for kymographic registration of respiration.

All substances were injected into the jugular vein through a plastic cannula (internal diameter 1.0 mm, length 3 to 4 cm). Drugs were injected in a volume of 1 ml/kg, and the cannula was washed after each injection with an equal volume of 0.9% NaCl. The following substances were used:

- N, N' — trimethylenebis (pyridinium-4-aldoxime) dichloride (TMB-4 Cl₂).
- 1-Phenacyloxime-quinolinium chloride (F-oxime).
- Oxotremorine
- Atropine sulphate, and
- Atropine methylnitrate.

TMB-4 Cl₂ and F-oxime were synthesized by Ing. M. Milojević in our Institute. Oxotremorine was kindly provided by Prof. P. Stern, Sarajevo. Atropine sulphate and atropine methylnitrate were obtained from commercial sources.

All the substances were dissolved in an isotonic solution of NaCl before each experiment.

The ECG was registered by means of an »Cardi-all« type electrocardiograph, using the second lead.

RESULTS

The i. v. injection of OTR, 0.1 mg/kg, caused an immediate transient fall of arterial blood pressure lasting 5 to 10 sec, followed by rise of arterial blood pressure reaching the maximum within one minute. Hypertensive effect of OTR was maintained at maximum level for more than 15 min. After that time, progressive decline of arterial blood pressure was registered, returning to control values 25 — 30 min after the injection. As shown in Table 1, the average increase of arterial blood pressure was 47 mm Hg. The height of hypertension was dose dependent, and 0.1 mg/kg of OTR produced submaximal effects.

OTR, 0.1 mg/kg, in addition to the effect on blood pressure, induced the following ECG changes: sinus bradycardia, reduction in the height of the P and T-wave. These changes were recorded only 2 to 3 min after the i.v. injection of OTR.

The results of the tested substances concerning the arterial blood pressure changes produced by OTR are presented in Table 1.

TABLE 1

EFFECTS OF TMB-4 Cl₂, F-OXIME, ATROPIN METHYLNITRATE
AND ATROPINE SULPHATE ON BLOOD PRESSURE IN RATS
TREATED WITH OXOTREMORINE

Drugs	Arterial blood pressure, mmHg (X $\pm$ SE)	
	A	B
Controls	68.1 $\pm$ 4.0	68.1 $\pm$ 4.0
Oxotremorine	115.7 $\pm$ 6.2*	117.0 $\pm$ 5.4*
TMB-4 Cl ₂	106.7 $\pm$ 5.1	122.0 $\pm$ 3.4
F-oxime	91.3 $\pm$ 4.2**	93.3 $\pm$ 3.8**
Atropinmethylnitrate	119.0 $\pm$ 7.3	115.0 $\pm$ 6.5
Atropin sulphate	82.5 $\pm$ 9.2**	62.5 $\pm$ 1.5**

Drugs

Column A = Tested drugs were given after oxotremorine.

Column B = Tested drugs were given before oxotremorine.

* P < 0.05 comparing with controls.

** P < 0.05 comparing with oxotremorine effects.

TMB-4 Cl₂, 10 or 20 mg/kg, had no influence on hypertension produced by OTR. When TMB-4 Cl₂ was injected before OTR, it failed to prevent or reduce the changes in arterial blood pressure produced by this substance.

On the other hand, F-oxime in a dose of 10 mg/kg, lowered the arterial blood pressure risen by OTR. This effect was obtained within 10 to 20 sec after the injection of F-oxime, and lasted more than 30 min. Given before OTR, F-oxime prevented the rise of arterial blood pressure (Table 1).

Atropin methylnitrate, in a dose of 2 mg/kg, had no influence on the hypertension produced by OTR; when injected before OTR, atropin methylnitrate failed to prevent or reduce hypertensive effect of this substance, but completely blocked hypotensive action of OTR. On the contrary, atropin sulphate, given in a dose of 2 mg/kg, either before or after OTR, completely blocked or returned to control values the changes in arterial blood pressure.

TMB-4 Cl₂ and F-oxime injected before OTR, lowered the intensity of bradycardia and abolished the other ECG disturbances produced by OTR. On the other hand, atropine sulphate or atropine methylnitrate, completely prevented bradycardia and the other ECG changes produced by OTR.

DISCUSSION

It can be seen from the results that the administration of OTR to rats caused initial transient fall in arterial blood pressure followed by hypertension. This finding suggests that, both, peripheral and central components of OTR actions, are responsible for the arterial blood pressure changes in rat. The changes in arterial blood pressure due to OTR could be prevented or blocked by atropine sulphate, while atropine methylnitrate was effective only in preventing hypotensive component. Inability of atropine methylnitrate to counteract the hypertensive effect of OTR, indicates that this hypertension is probably central in origin.

In our experiments, TMB-4 Cl₂ was unable to prevent or abolish the increased arterial blood pressure caused by OTR. On the other hand, previous findings that bispyridinium oximes (TMB-4 Cl₂ and others) could abolish cardiovascular changes, including hypertension, in rats poisoned by certain organophosphorus compounds, was suggested as direct antiacetylcholine action of oximes (6, 9, 10, 12, 13, 16). The diversity in TMB-4 Cl₂ actions found in our experiments and those described in poisoning by anti-ChE agents, could probably be explained by different mechanisms responsible for the hypertension in rat induced by OTR or anti-ChE agents, respectively.

In opposite to TMB-4 Cl₂, F-oxime lowered the arterial blood pressure raised by OTR. Since both oximes are quaternary ammonium compounds, it is expected that the rate of their penetration into the central nervous system is very similar. However, in relation to their peripheral action on the cardiovascular changes caused by OTR, both oximes were equally effective.

The greater effectiveness of F-oxime in relation to TMB-4 Cl₂ suggests that the former substance has some additional anticholinergic (probably atropine-like) actions, independently of whether these effects are directed to the centre or periphery of the nervous system (ganglia, etc.). This point of view is supported by the finding that an oxime very similar in structure to F-oxime (POMP-4) was, concerning ganglion-blocking activity, twice as effective as TMB-4 Cl₂ (13).

Unfortunately, our preliminary results are insufficient to furnish a more complete pharmacological analysis of TMB-4 Cl₂ and F-oxime in relation to pharmacodynamic effects of OTR.

SUMMARY

The administration of OTR (0.05—0.1 mg/kg intravenously) to anaesthetized rats resulted in an immediate, transient fall in arterial blood pressure, which after 5 to 10 sec was followed by an increase of arterial blood pressure. According to the dose used, an increased arterial blood pressure lasted from 25 to 30 min. The fall of arterial blood pressure was invariably accompanied by pronounced bradycardia, and by the reduction in the height of the P and T-wave.

The oxime TMB-4 Cl₂ was ineffective in preventing or abolishing the changes of arterial blood pressure caused by OTR. F-oxime, was relatively effective in counteracting the changes in arterial blood pressure induced by OTR. Both oximes, given before OTR, lowered the intensity of bradycardia and abolished ECG disturbances.

Atropine methylnitrate, given before OTR, completely prevented hypotension, but was unable to prevent or abolish the hypertensive action of OTR. Atropin sulphate, completely prevented or abolished changes in arterial blood pressure caused by OTR. Both, atropine methylnitrate and atropine sulphate effectively prevented bradycardia and the other ECG changes produced by OTR.

B. BOŠKOVIĆ, N. ROSIĆ I V. VOJVODIĆ

NEKA FARMAKOLOŠKA DJELOVANJA OKSIMA I HOLINOLITIKA NA KARDIOVASKULARNE EFEKTE IZAZVANE POMOĆU OKSOTREMORINA NA ANESTETIZIRANIM ŠTAKORIMA

KRATAK SADRŽAJ

Administracija OTR (0,05 — 0,1 mg/kg i. v.) na anesteziranim štakorima je prouzrokovala neposredan, tranzitorni pad arterijskog krvnog pritiska, iza čega je, poslije 5 do 10 sec., slijedio njegov porast. Zavisno od upotrijebljene doze, povišeni arterijski krvni pritisak je trajao od 25 do 30 min. Pad arterijskog krvnog pritiska je u svim slučajevima bio praćen izraženom bradikardijom i smanjenjem visine P- i T-talasa.

Oksim TMB-4 Cl₂ nije mogao da spriječi promjene arterijskog krvnog pritiska prouzrokovane sa OTR. F-oksime je imao nekog efekta u ovom smislu. Ako se oba oksima daju prije OTR smanjuju intenzitet bradikardije i sprečavaju poremećaje u ECG.

Atropin metilnitrat, ako se dâ prije OTR, sprečava pojavu hipotenzije, ali nije u stanju da suzbije njegovo hipertenzivno djelovanje. Atropin sulfat je potpuno spriječio promjene arterijskog krvnog pritiska izazvanog sa OTR. Oba atropina, metilnitrat i sulfat, spriječili su nastajanje bradikardije i druge ECG promjene izazvane sa OTR.

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SOME PHARMACOLOGICAL ACTIONS OF AMANTADINE HYDROCHLORIDE AND ITS EFFECTS ON EXPERIMENTAL AND CLINICAL TREMOR

INTRODUCTION

Amantadine hydrochloride was first introduced as an antiviral agent effective against A₂ influenza (8, 9). In subsequent use it was found to have a beneficial effect in Parkinson's disease (11, 15). The work to be reported is a general examination of the pharmacology of amantadine and also its application in the treatment of essential tremor in man.

METHODS

(i) Isolated guinea-pig ileum preparation.

Segments of ileum 2.5 cm in length were suspended in Krebs solution maintained at $37 \pm 1^\circ\text{C}$ bubbled with a mixture of 95% O₂ and 5% CO₂. Tension changes were recorded isometrically. The initial tension applied to the ileum was 1g.

(ii) Isolated rat and rabbit preparations.

Isolated rat uteri were set up as described by Gaddum and Lembeck (5), except that isometric rather than isotonic recordings were made.

Spiral strips of rabbit aorta were set up as described by Furchgott and Bhadrakom (4) except that isometric recording techniques were used.

Isolated perfused rat and rabbit hearts were set up for force and rate recordings (1).

(iii) Effect of amantadine on drug induced tremor in mice.

The dose of tremorgenic agent producing tremor in 50% of a group of mice treated with the drug was determined by observation using a »double-blind« technique. The results were calculated according to the method of Litchfield and Wilcoxon (10).

(iv) Clinical Trials.

Patients diagnosed as essential tremor were asked if they would participate in a clinical trial. The diagnosis was based on criteria for this disease laid down by Critchley, 1949 (2).

The patients' ages ranged from 43—67 years and patients of both sexes were included in the trial. Double blind conditions were observed and clinical assessments made whilst the patients were on placebo or on drug. The clinician ranked improvement or deterioration on a ranking scale ranging from —3 to +3. —3 represented marked deterioration whilst +3 represented marked improvement.

A number of tests were carried out. The patient was presented with 6 wooden blocks (each side of the cube 2.5 cm) and asked to arrange 3 blocks on the bottom row, 2 blocks in the middle row and one on top. The time taken to perform this exercise was noted. The time taken for the patient to fold a piece of paper and place it in an envelope was noted as well as the time taken to open and close a safety pin. The patient was asked to trace round the outline of a 3 cm diameter circle. The time taken was noted. In addition a score of 1 to 3 was given for closeness to the outline, 1 being very close, 2 following the outline moderately well and 3 marked excursions from the outline. The time taken to perform this exercise was multiplied by the 'closeness' factor.

SCHEMATIC DIAGRAM FOR TREMOR RECORDING

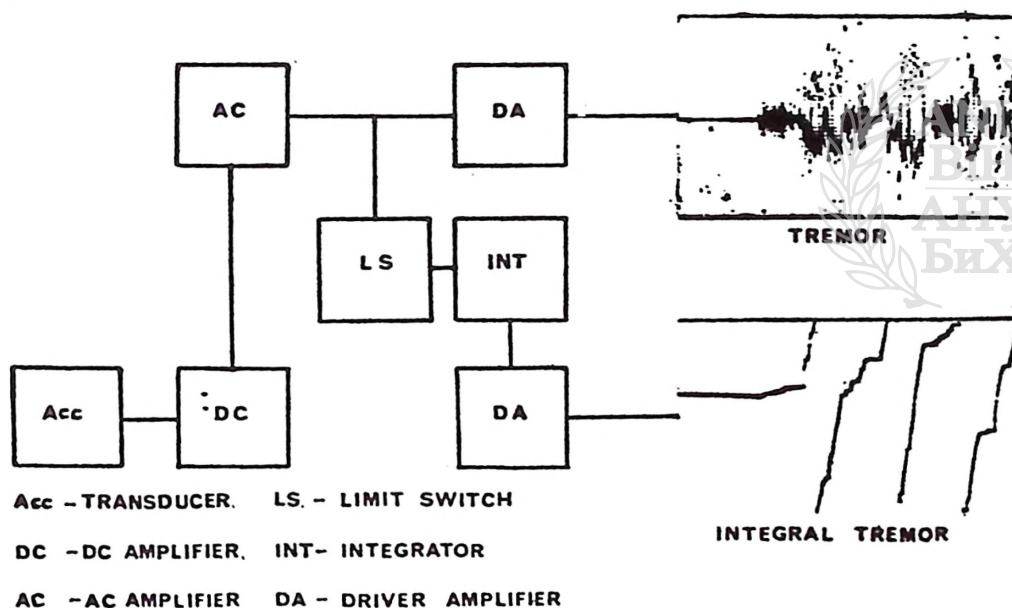


Fig. 1

Schematic diagram for tremor recording.

ACC Grass accelerometer SPA 1
 DC DC amplifier Devices DC2D
 AC AC amplifier Devices AC 1
 DA Driver amplifiers DC5 (M2 Devices Recorder)
 LS Limit Switch
 INT Integrator } Devices 3020

A recording of tremor and integral of tremor is shown. The integrator pen is automatically zeroed on reaching full deflection.

Similarly the time taken to print a phrase was noted and a ranking score of 1—3 (1 very neat) was given. The time taken was multiplied by the 'neatness' factor. The patients was also instructed to remove a match from a box of matches and strike it and time taken was noted.

In addition tremor was estimated using a Grass Parkinsonian transducer, the output of which was fed through amplifiers so that tremor trace and integral of tremor could be obtained (fig. 1). All patients were allowed a pre-trial run on the tests described above and a pre-trial tremor recording was taken so that they could get acclimatised to the procedure. The results were analysed using non-parametric statistics (6).

DRUGS USED

We would like to thank Dr. A. W. Galbraith, Geigy Pharmaceuticals Ltd., Macclesfield, Cheshire, England for kindly supplying amantadine hydrochloride, and Dr. R. W. Brimblecombe, Ministry of Defence Chemical Defence Establishment, Porton Down, Wiltshire, England for generously donating some oxotremorine pure substance. Other drugs used were: Acetylcholine (Acecoline, Lematte et Boinot, Paris), bradykinin, Sandoz, Ltd., carbachol chloride and histamine phosphate (B. D. H. Ltd.), dopamine hydrochloride, harmine hydrochloride, homovanillic acid, 5-hydroxyindole-3-acetic acid, 5-hydroxytryptamine, noradrenaline bitartrate (Koch-Light Ltd.), propranolol hydrochloride (I. C. I.) and tremorine (Sigma Chemicals Ltd.)

RESULTS

(i) Isolated guinea-pig ileum preparation.

The effect of Amantadine hydrochloride on guinea-pig ileum is shown in Table 1. Amantadine in concentrations of 5 µg/ml or less

Table 1

INTERACTION BETWEEN AMANTADINE AND SOME AGONISTS ON THE ISOLATED GUINEA-PIG ILEUM PREPARATION

Agonist	Amantadine µg/ml				
	5	10	40	160	640
None	0	+	++	+	—
Acetylcholine	0	+	++	0	—
Bradykinin	0	++	+	0	—
5-HT	+	++	+	0	—
Histamine	+	+	++	0	—

0 = no effect.

+ = potentiation or increased activity.

— = antagonism or decreased activity.

Minimum number of observations for each concentration = 5.

was without effect on the resting tension. A concentration of 10 $\mu\text{g/ml}$ induced small rhythmic tension changes in some segments. All segments tested showed this response at 40 $\mu\text{g/ml}$, and the effect persisted up to a concentration of 160 $\mu\text{g/ml}$. At higher concentrations the ileum became quiescent. Table 1 also shows the effect of amantadine on the response of the ileum to various agonists. A dose response curve was established for acetylcholine and repeated in the presence of various concentrations of amantadine, five separate experiments being made at each concentration. With 5 $\mu\text{g/ml}$ amantadine hydrochloride no change occurred in the dose response curves. At 10 and 40 $\mu\text{g/ml}$ all the dose response curves were shifted to the left indicating potentiation, no leftward shift was apparent at 160 $\mu\text{g/ml}$. The ileum did not respond to acetylcholine added in the presence of amantadine 640 $\mu\text{g/ml}$. Segments of ileum were also tested with ED 70 doses of bradykinin, 5-hydroxytryptamine and histamine (table 1) similar results to those with acetylcholine were obtained.

(ii) Isolated rat and rabbit preparations.

Table 2 shows the effect of amantadine alone on some isolated tissue preparations and the effect of amantadine on responses of these tissues to dopamine and noradrenaline.

Table 2
EFFECT OF AMANTADINE ON SOME ISOLATED RAT
AND RABBIT TISSUES

	Minimum effective concentration ⁺ of Amantadine (g/ml)		
	Alone	With Dopamine	With Noradrenaline
Rat Uterus	0, 4×10^{-5}	+, 1×10^{-5}	+, 4×10^{-5}
Rabbit Aorta	0, 1×10^{-3}	0, 1×10^{-4}	0, 1×10^{-4}
Rabbit Heart	+, $2 \times 10^{-7*}$	0, 1×10^{-5}	0, 1×10^{-5}
Rat Heart	+, $2 \times 10^{-7*}$	0, 1×10^{-5}	0, 1×10^{-5}

0 = no effect.

+ = potentiation or increased activity.

All other figures represent concentration of amantadine hydrochloride in Krebs, except at* where figures represent dose injected in a volume of 0.2 ml. For significance of changes see text. + Minimum effective concentration when amantadine active; when inactive figures represent maximum concentration used.

Amantadine alone was without effect on the uterus in concentrations up to 4×10^{-5} g. Concentrations higher than this reduced the response of the uterus to carbachol. Dopamine was used as an inhibitor of the carbachol spasm and its inhibitory effect measured in the presence and absence of amantadine slight potentiation of dopamine was observed with amantadine 10 $\mu\text{g/ml}$, which became significant ($p < 0.05$ Mann-Witney 'U' test) (6) when the concentration was increased to 4×10^{-5}

g/ml. When noradrenaline was used as the inhibitor of the carbachol spasm, slight non-significant potentiation was observed only at 4×10^{-5} g/ml amantadine.

Amantadine in concentrations as high as 10^{-3} g/ml was without effect on the resting tension of spiral strips of rabbit aorta. Amantadine hydrochloride 10^{-4} g/ml in the Krebs solution was without effect on cumulative log dose-tension response curves obtained with either dopamine or noradrenaline.

The effect of amantadine on isolated heart preparations is also shown in Table 2. Some hearts (rat, 3 of 13 tested, rabbit, 1 of 6 tested) showed increased force of contraction but no change in rate of beating with a dose of 2×10^{-7} g amantadine hydrochloride. The increased force response was blocked by propranolol. The remaining hearts tested did not respond to amantadine until doses of 2×10^{-4} g were used, when there was usually a decrease in heart rate. Amantadine (1×10^{-5} g/ml) was added to the perfusing Krebs solution and was without effect on either dopamine or noradrenaline induced rate and force changes (6 experiments).

Table 3
EFFECT OF AMANTADINE ON DRUG INDUCED TREMOR IN MICE

TREMOR DRUG	PRETREATMENT	
	SALINE 0.9%	AMANTADINE 100 mg/kg
TREMORINE TD 50	7.2 mg/kg	4.2 mg/kg
Confidence limits	5.3—9.7	3.0—5.9
Number of mice	60	59
OXOTREMORINE TD 50	0.18 mg/kg	0.04 mg/kg
Confidence limits	0.15—0.22	0.025—0.06
Number of mice	110	60
HARMINE TD 50	5.8 mg/kg	1.8 mg/kg
Confidence limits	3.9—8.7	1.1—2.9
Number of mice	40	40

TD 50 = dose of tremor-drug producing tremor in 50% of mice treated calculated according to Litchfield & Wilcoxon (1949) (10).

(iii) Effect of amantadine on drug-induced tremor in mice.

Table 3 shows the effect of amantadine hydrochloride 100 mg/kg on drug induced tremor in mice. The dose of tremorgenic agent producing tremor in 50% of the mice treated (TD 50) was determined (10). The TD 50 for tremorine, oxotremorine and harmine was reduced by pretreatment of the mice with amantadine. In each case the potentiation was significant at the 95% confidence limits.

A preliminary trial was carried out in which nine patients received 100 mg daily of amantadine hydrochloride for 1 week followed by 200 mg daily for 5 weeks. 8 other patients received placebo over the

same period. Clinically no significant improvement occurred as compared to placebo. 3 patients in each group showed some evidence of improvement over their base line performance. There was no significant improvement in recorded tremor.

A double blind crossover trial using higher doses of amantadine hydrochloride for a similar period was then conducted. Ten patients received one capsule daily during the first week of drug (100 mg) or placebo, 2 capsules daily the second week and 3 capsules daily on subsequent weeks. On comparing results at the end of the placebo period with the end of the amantadine period, 5 patients clinically scored better on drug than placebo and 2 scored better on placebo than drug. 3 others had equal scores. The scores on drug were higher than on placebo and were significant ($p < 0.05$ signed Rank test one tail) (6).

The result of the tests are shown in table 4. It will be seen that the patients ability to fold paper improved but their ability to remove

Table 4

TEST	TREND IN FAVOUR OF	SIGNIFICANCE*
BLOCKS	DRUG	$p > 0.05$
FOLDING PAPER	DRUG	$p < 0.01$
SAFETY PIN	DRUG	$p > 0.05$
CIRCLE	PLACEBO	$p > 0.05$
SENTENCE	DRUG	$p > 0.05$
TREMOR	DRUG	$p < 0.05$
MATCH	PLACEBO	$p < 0.05$

* Wilcoxon signed Rank Test. (Two tailed test).

and strike matches did not. A significant diminution in tremor occurred (fig. 2). The trends were mainly in favour of the drug and not of the placebo.

Among the patients'; reports of improvement, one patient reported he could now shave with a safety razor and another that she could dress herself.

Side effects were noted by a number of patients. Sleep problems were the commonest complaints on the drug. These included dreaming nightmares, early wakening and difficulty in getting to sleep (three patients). One patient complained of dizziness and one had palpitations. No serious side effects were seen. On placebo, one patient complained of dizziness, two had sleep problems, difficulty in getting to sleep or dreaming and two complained of chest pain.

Some determinations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5 HIAA) were made from samples of cerebrospinal fluid (C. S. F.) taken from patients with Parkinson's disease (3). Wide variation was found from patient to patient. No significant difference could be detected between samples taken during treatment with amantadine when compared with samples taken whilst the patients were receiving L-dopa.

The minimum number of samples estimated was 8. In a number of patients 2 samples were taken from the same person once during amantadine therapy and once during L-dopa medication. Nine paired samples were examined for 5 HIAA content. Five patients had higher 5 HIAA/CSF levels during the amantadine period and four had higher CSF levels

PLACEBO

AMANTADINE

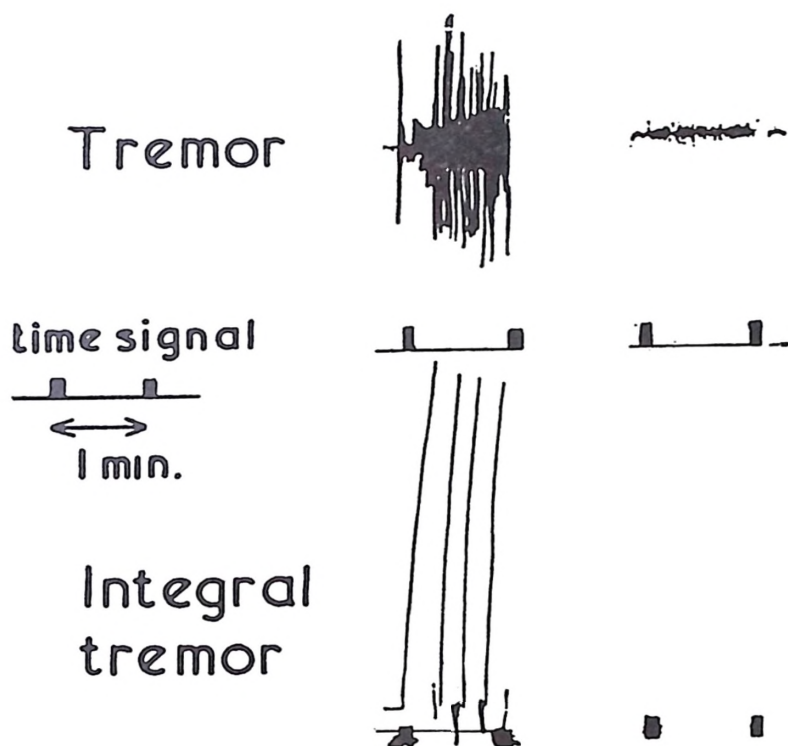


Fig. 2

Tremor recording. Patient on 300 mg amantadine daily or placebo. Recording taken at end of the placebo period or period on amantadine. Tremor was recorded for 60 seconds.

during L-dopa therapy. Eight paired samples were also obtained for HVA. In this case 5 patients had higher CSF levels during amantadine therapy, two patients had higher CSF levels during L-dopa treatment. No HVA could be detected in either sample taken from the remaining patient. When examined statistically using the Wilcoxon matched pairs signed rank test (6), the differences in HVA content of CSF just failed to achieve statistical significance ($p < 0.05$). The difference in 5 HIAA content of CSF also failed to achieve the accepted level for statistical significance. Both tests being »one-tailed tests«.

DISCUSSION

Experiments on isolated tissues indicated that amantadine had very little activity on its own. Also its interaction with various agonists on the guinea-pig ileum was non-specific, causing potentiation at low concentrations and antagonism at high concentrations. A non-specific blockade of histamine and acetylcholine on guinea-pig ileum has been reported previously (7). Of all the tissues tested only on the rat uterus did amantadine show a significant potentiation of a dopamine effect. There was also a slight potentiation of noradrenaline on this tissue.

Amantadine was found to potentiate drug-induced tremor in mice. Since it has been shown to alleviate the Parkinsonian condition in man (11, 15), these findings must throw more doubt on the usefulness of tremorgenic drugs as models of Parkinson's disease.

On the objective tests in patients, one would have expected that an improvement on the folding paper test which is a test of manual dexterity would have been accompanied by a similar improvement on the match test which again tests dexterity in handling objects. However it should be noted that patients on placebo took a mean time of 6.6 sec to complete this test whilst the mean time for the folding paper test was 16.2 sec. The reaction time of the observer using the stopwatch would therefore be of greater importance in the first instance than in the second and this factor of observer error may therefore have been of importance in producing the result obtained in the match test.

There have been a number of clinical trials of amantadine in Parkinsonism and Parkinsonian tremor has been reported to be improved by the drug (13, 15). Schwab, England, Poskanzer and Young (15) state the drug is of no benefit in familial tremor but do not give details. Partera, Varala de Seijas, Mara, Ramírez Echevarría and Amaya Pembo (14) treated 9 patients with senile tremor with 300 mg per day of amantadine for 25 days. They observed no improvement. Though the dose was the same as in the present series the duration of treatment was less. Parkes, Knill-Jones and Clements (12) report that the familial tremor of 2 female patients was exacerbated by amantadine 300 mg daily. The duration of treatment is not noted.

A significant change in response is not necessarily of therapeutic importance as it is the degree of improvement that matters. In the present series clinically in some patients improvement occurred and this was borne out by the patients subjective impressions. On the basis of this small trial it would appear that amantadine is of some benefit in this disease though if one takes into account side effects the benefits maybe only marginal.

In Parkinsonian patients it was not possible to detect any significant change in the concentrations of HVA and 5 HIAA in CSF samples taken during amantadine therapy when compared with patients on L-dopa. This was probably due to the wide individual variations in the CSF content of these substances. When the results for patients giving two CSF samples were extracted from all the results obtained, then a

slightly different pattern emerged. For the majority of patients (5 out of 8) there were lower concentrations of HVA in the CSF samples taken during the L-dopa period than the concentrations found whilst the same patients were receiving amantadine. One possible explanation for this finding is that amantadine is increasing the release and conversion of dopamine to its acid metabolite. A decrease in the concentrations of 5 HIAA in CSF of Parkinsonian patients receiving L-dopa has been reported previously (16). In our trial though not significant the mean 5 HIAA concentration was lower for CSF taken during L-dopa therapy than the concentration during amantadine therapy.

SUMMARY

Amantadine hydrochloride was tested for its effect on isolated guinea-pig ileum, rat uterus, rabbit aortic strip and rat or rabbit perfused heart. Amantadine on its own produced small rhythmic tension changes in the guinea-pig ileum in concentrations of 40 $\mu\text{g/ml}$. It was without effect on rat uterus and rabbit aorta, but had a variable action on perfused hearts. Some hearts showed an increased force of contraction at a dose of 2×10^{-7} g/ml but the majority failed to respond in this way even at 2×10^{-4} g/ml. Its interaction with various agonists on the guinea-pig ileum suggests it is a non-specific potentiating agent at low doses (40 $\mu\text{g/ml}$) and a non-specific inhibitor at high doses (640 $\mu\text{g/ml}$). Amantadine did not potentiate either dopamine or noradrenaline on rabbit aorta and rat or rabbit heart. It did however increase the ability of dopamine and noradrenaline to inhibit carbachol spasms of rat uterus. Amantadine potentiated the tremorgenic actions of tremorine, oxotremorine and harmine.

A double blind crossover trial of 10 patients with essential tremor was carried out using amantadine hydrochloride and placebo. 300 mg of amantadine hydrochloride produced some slight improvement and this was borne out by tremor recordings. The drug produced some side effects as did the placebo.

No significant difference could be detected in the HVA and 5 HIAA content of CSF taken from Parkinsonian patients receiving either L-dopa or amantadine.

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**NEKA FARMAKOLOŠKA DJELOVANJA AMANTADINA
I NJEGOVI EFEKTI NA TREMOR**

KRATAK SADRŽAJ

Rad se odnosi na istraživanja raznih aspekata farmakologije amantadina. Inicijalni eksperimenti odnosili su se na upotrebu izoliranih tkiva i takođe na neke in vivo eksperimente sa mišem. Daljni eksperimenti odnose se na evaluaciju terapeutske efikasnosti amantadina na izvjesna čovjekova tremor-stanja.

Determiniran je efekat amantadina na izvjestan broj izoliranih preparata tkiva (npr. ileum zamorčeta, uterus štakora, aorte zeca i izolirano perfuzirano srce). Ista ova izolirana tkiva upotrijebljena su za ispitivanje interakcije između amantadina i nekih transmitterskih supstanci. Ispitivano je i dještvo amantadina na tremor miša izazvan pomoću farmaka.

Vršena su klinička ispitivanja djelovanja amantadina na pacijente sa esencijalnim tremorom. Tremor je praćen objektivima i pacijenti su učestvovali u izvjesnom broju testova čiji je cilj bio da se pokaže da li lijek izaziva bilo kakvo poboljšanje na sposobnost izvođenja finih pokreta. Prikupljeni su CSF-uzorci od nekoliko pacijenata i koncentracije homovanilične kiseline i 5-hydroxyindolactone kiseline mjerene za vrijeme dok su pacijenti uzimali ili placebo ili aktivni lijek.

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DISCUSSION

Jurna: Did I understand you correctly when you said that the sensitivity of aortic strips towards NA or DA was not increased by amantadine?

Cox: The action of amantadine was determined on the aortic strip preparation in the following way. Cumulative dose response curves were obtained to noradrenaline and dopamine, and then repeated in the presence of amantadine concentrations up to those shown on the slide. Amantadine failed to potentiate either noradrenaline or dopamine.

Biochemical changes



I. JURNA

DRUG INDUCED RIGIDITY AND TREMOR IN THE RAT

During the last years much evidence has been accumulated in favour of the view first formulated by Barbeau (1962) that the motor symptoms in Parkinson's disease may result from an imbalance between monoaminergic and cholinergic mechanisms of the brain. Hornykiewicz and coworkers (Ehringer and Hornykiewicz, 1960; Birkmayer and Hornykiewicz, 1964) found a marked reduction in the content of monoamines, especially dopamine, in the neostriatum of Parkinson patients. Accordingly, application of L-DOPA as the precursor of dopamine alleviates the extrapyramidal motor syndrome (Barbeau et al., 1962; Birkmayer and Hornykiewicz, 1961, 1962). On the other hand, reduced monoaminergic activity may also be compensated by drugs with cholinolytic properties, as for instance the classical antiparkinson drug atropine.

Under experimental conditions a parkinson-like state of motor disturbance is induced by drugs which either depress monoaminergic (dopaminergic or noradrenergic) or increase cholinergic activity. Impairment of monoaminergic transmission may result from a depletion by reserpine or tetrabenazine of monoamines in the brain (Carlsson et al., 1957; Quinn et al., 1959; Bertler, 1961; Pletscher et al., 1962; Carlsson, 1966) or from block of the respective receptor sites by chlorpromazine, haloperidol or phenoxybenzamine. Enhancement of cholinergic transmission will follow from an inhibition by physostigmine of cholinesterase activity. In the rat the application of each of the drugs mentioned produces rigidity, tremor and akinesia (Steg, 1964; Roos and Steg, 1964; Arvidsson et al., 1966; Jurna et al., 1969). Since drugs interfering with monoaminergic transmission are also known to be able to develop a Parkinson syndrome in man, they might be considered as useful tools for the study of changes in motor activity underlying this type of extrapyramidal disorder, and to test drugs on a possible antagonistic action.

The following part deals with the results obtained when investigating two of the main motor symptoms in experimental parkinsonism, that is rigidity and tremor.

Rats were operated under halothane anaesthesia. The spinal cord was exposed, the dorsal roots supplying the calf muscles were prepared

for electrical stimulation, and thin filaments were isolated from the corresponding ventral roots, care being taken that their greater part was left unsevered. The animals were fixed rigidly and the spinal cord was covered with warm paraffin oil. All drugs were injected into one of the tail veins.

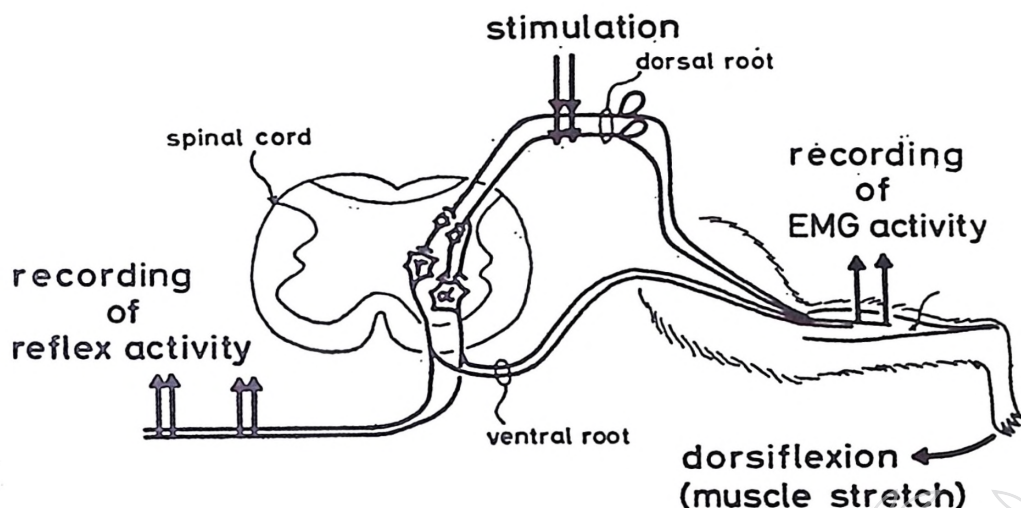


Fig. 1

Schematic representation of the experimental situation

Fig. 1 illustrates the experimental situation. The dorsal root was mounted on a pair of stimulating electrodes. A ventral root filament was placed on two pairs of recording electrodes, one pair being situated closest to the spinal cord, the other more distally. The distance between the two pairs of recording electrodes was about 5 mm. The activity recorded from the ventral root filaments was displayed on a dual beam oscilloscope, where the upper and lower beam presented the potentials led off with the proximal and distal pairs of electrodes respectively. In this way discharges from alpha and gamma motoneurons could be identified by differences not only in shape and amplitude, but also in conduction velocity of the potentials. Reflex discharges were elicited by stimulation of the corresponding dorsal root with single rectangular pulses of supramaximal strength. Electromyographical activity was recorded from the calf muscles at rest and during stretch produced by dorsiflexion of the foot.

In the untreated rat alpha reflex discharge is very low and gamma activity is high (Fig. 2). After the administration of a high dose of reserpine (10 mg/kg) alpha activity increases markedly and gamma activity is depressed (Steg, 1964; Roos and Steg, 1964; Arvidsson et al., 1966). Moreover, reserpine reduces the latency between the stimulus artifact and the first alpha reflex response (Jurna and Lanzer, 1969). Simultaneously with the increase in alpha motor activity rigidity develops manifesting itself by tonic activity in the electromyogram during sustained muscle stretch.

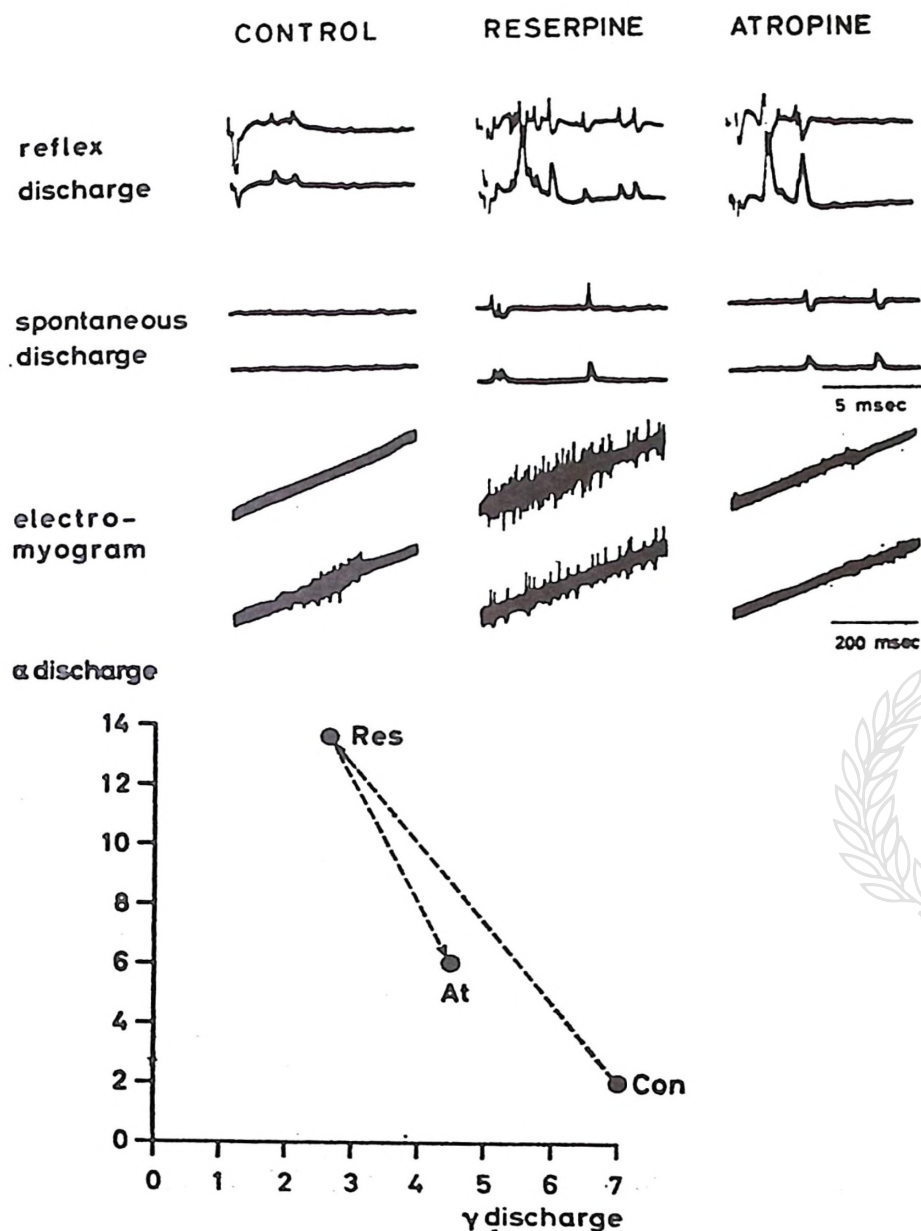


Fig. 2

Effect of reserpine and atropine on motor control. Upper row of recordings: reflex discharge led off from ventral root filament following stimulation of corresponding dorsal root with single impulse. Middle row of recordings: spontaneous discharge from ventral root filament. Upper tracings: recording made with electrode proximal to spinal cord; lower tracings: recording made with distal electrode. Recordings made with running beam on stationary film. — Lower row of recordings: electromyographical activity led off from the calf muscle. Recordings made with running beam on running film. — First column of recordings: control recordings showing little alpha but high gamma

Similar results have been obtained with other drugs impairing monoaminergic transmission, such as chlorpromazine, haloperidol, phenoxybenzamine or tetrabenazine, and also with physostigmine (Roos and Steg, 1964; Arvidsson et al., 1966; Jurna et al., 1969). Therefore, parkinson-like rigidity induced in the rat is of the alpha type, in contrast to rigidity resulting from intercollicular decerebration, which is of the gamma type. Interestingly enough, chlorpromazine has been shown to reduce gamma activity also in the decerebrate preparation (Henatsch and Ingvar, 1956).

Drugs which either stimulate monoaminergic or depress cholinergic activity abolish rigidity and normalize the disturbed control of spinal motor activity in the rat (Roos and Steg, 1964; Arvidsson et al., 1966; Jurna and Lanzer, 1969; Jurna et al., 1969). This is achieved with typical anti-parkinson drugs as atropine (Fig. 2), biperiden, trihexyphenidyl and caramiphen, or with L-DOPA (Fig. 3) or metamphetamine.

The similarity between experimental rigidity and the corresponding clinical symptom in Parkinsonism is furthermore stressed by the observation that rigidity is abolished by removal of the striatum, but not by decortication, ablation of the cerebellum, lesion of the vestibular and red nuclei, nor by pyramidectomy (Arvidsson et al., 1967).

Another group of drugs which has found widespread application in the study of substances on possible effectiveness in Parkinson's disease, although as yet almost exclusively with respect to suppression of tremor activity, consists of tremorine and its active metabolite oxotremorine. Both tremorigenic agents have been reported to produce tremor and spasticity in various animal species (Everett, et al., 1956; George et al., 1962). They raise the concentration of acetylcholine in the brain (Holmstedt et al., 1963; Holmstedt and Lundgren 1964; Lundgren and Malmberg 1968) and could therefore be expected to evoke changes in spinal motor control similar to those following the application of physostigmine.

In fact, tremorine and oxotremorine induce a state of marked rigidity in the rat which is signalled by the appearance of tonic activity in the electromyogram (Fig. 4) and an increased resistance against dorsiflexion of the foot. At the same time alpha reflex activity is increased, the latency between the stimulus artifact and the first alpha discharge is shortened, and spontaneous bursts of alpha discharges can be observed (Jurna et al., 1970). Moreover, rigidity and alpha hyper-

reflex discharge, spontaneous gamma activity and phasic activity in the electromyogram during quick stretch of muscle. Second column of recordings: recordings made 30 min after the injection of reserpine 10 mg/kg, showing high alpha and low gamma activity, shortening of the interval between stimulus artifact and first alpha discharge, spontaneous alpha activity, and tonic activity during sustained muscle stretch. Third column of recordings: recordings made 40 min after the injection of atropine 5 mg/kg, showing depressin of alpha and electromyographical activity. — The diagram represents the mean values of the number of alpha and gamma reflex discharges per sweep during the control period Con, after reserpine Res, and after atropine At. (from Jurna and Lanzer, 1969).

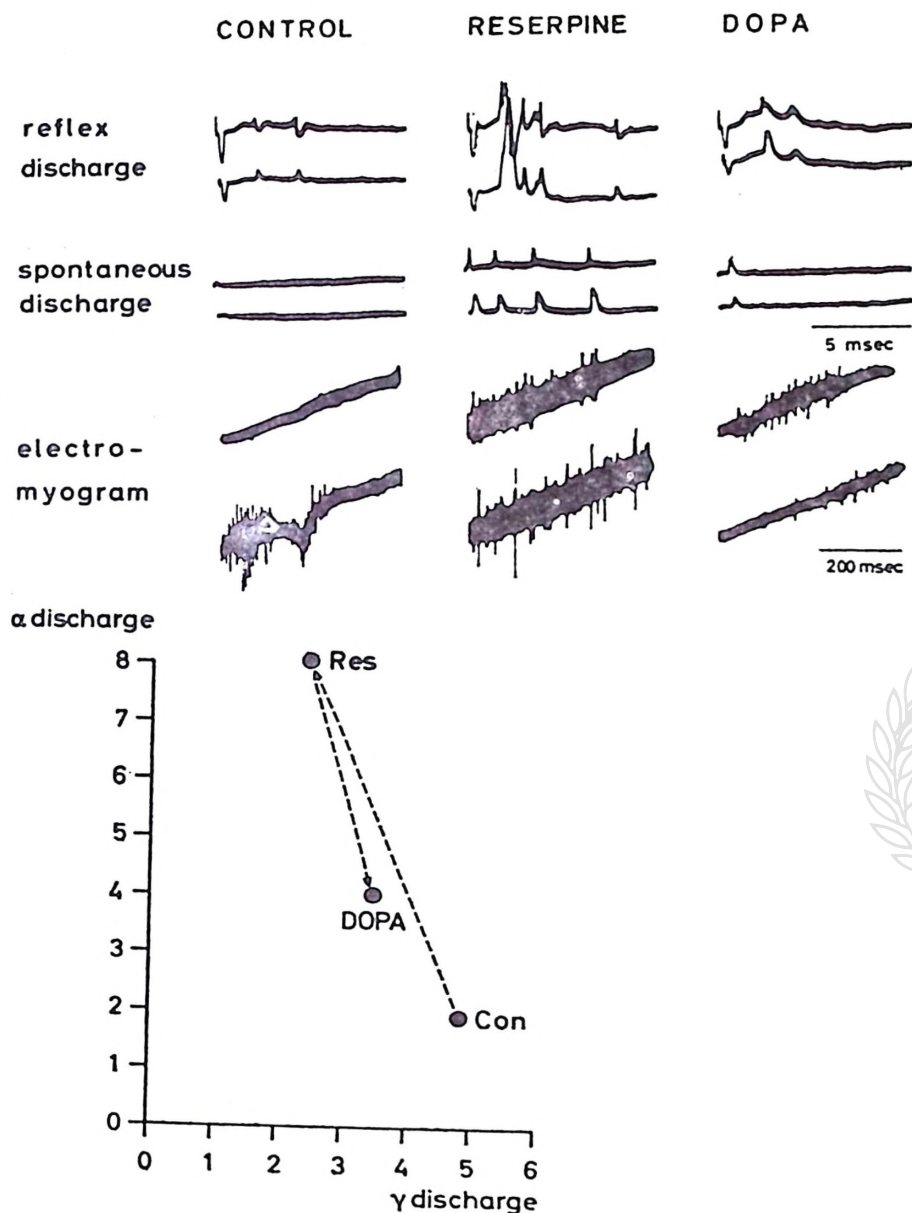


Fig. 3

Effect of reserpine and Dopa on motor control. Recordings as in Fig. 2. The record represented in the third column were taken 40 min after the injection of Dopa 100 mg/kg. The diagram represents the mean values of the number of alpha and gamma reflex discharges per sweep during the control period Con, after reserpine Res, and after Dopa. (from Jurna and Lanzer 1969).

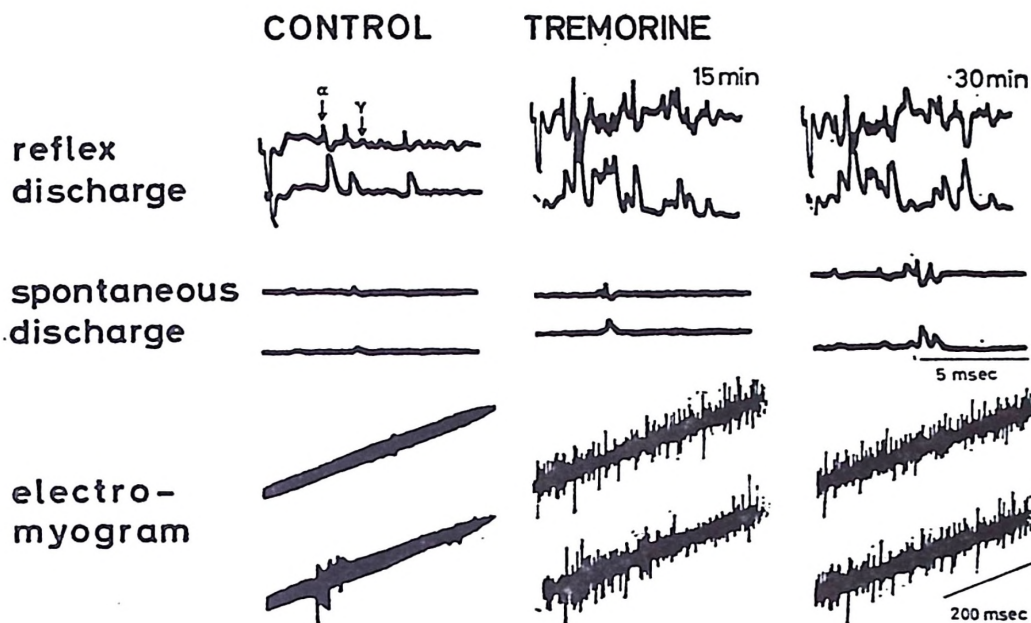


Fig. 4

Effect of tremorine on alpha and gamma motor discharges and electromyographic activity. Recordings as in Fig. 2. First column of recordings: control recordings showing little alpha and high gamma reflex discharge (an alpha and a gamma discharge are indicated by arrows), spontaneous gamma activity and phasic activity during quick muscle stretch. Second and third column of recordings: recordings made 15 and 30 min after the injection of tremorine 10 mg/kg, showing increased alpha reflex activity, shortening of the interval between stimulus artifact and first alpha discharge, spontaneous alpha discharges and increased spontaneous gamma discharges, and tonic activity in the electromyogram. (from Jurna et al., 1970).

activity from tremorine or oxotremorine are depressed by antiparkinson drugs with cholinolytic properties (Fig. 5) which have been shown to antagonize also reserpine and physostigmine rigidity.

Thus, as far as experimental rigidity is concerned, the changes in motor control produced by reserpine, physostigmine and tremorine or oxotremorine are identical.

With respect to the generation of tremor activity, however, the situation is different. Reserpine as well as tremorine or oxotremorine produce tremor activity, but obviously in different ways. As has been mentioned before, reserpine stimulates spontaneous alpha activity and depresses gamma activity. Therefore, tremor resulting from the administration of a high dose of reserpine must be operated by the alpha motor system which may be found to discharge in a burst-like fashion (Fig. 6) Similar bursts of alpha activity are also observed after an application of metamphetamine.

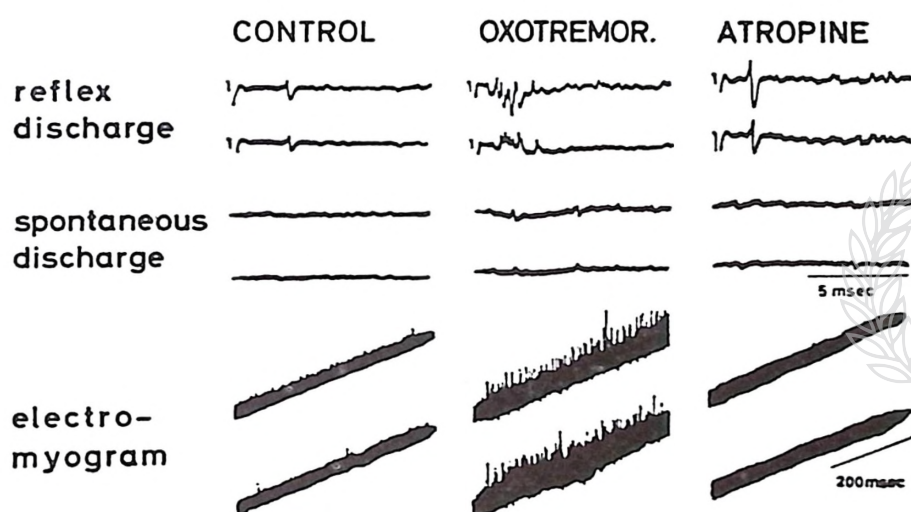
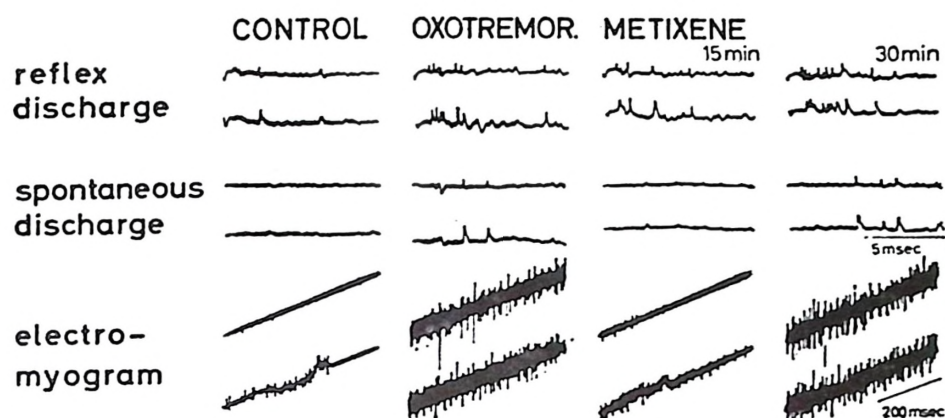


Fig. 5

Effect of oxotremorine on motor control and its depression by metixene and atropine. Both sets of recordings as in Fig. 2. First column: control recordings. Second column: recordings made 15 min after the begin of the infusion of oxotremorine 40 μ g/kg/min.

Following columns: recordings made 15 min and 30 min after the injection of metixene 1 mg/kg or 15 min after the injection of atropine 5 mg/kg. (from Jurna et al., 1970).

Tremorine and oxotremorine, on the other hand, do not depress but increase the spontaneous activity of the gamma motoneurons which discharge in bursts. Spontaneous alpha activity may also appear after tremorine or oxotremorine, but this is probably due to an activation by way of the so called gamma loop (gamma motoneurone —

muscle spindle — afferent fibre — alpha motoneurone; Granit 1955). The importance of the gamma motor system in tremorine and oxotremorine tremor is confirmed by the observation that drugs depressing the gamma motor hyperactivity induced by both tremorigenic agents,

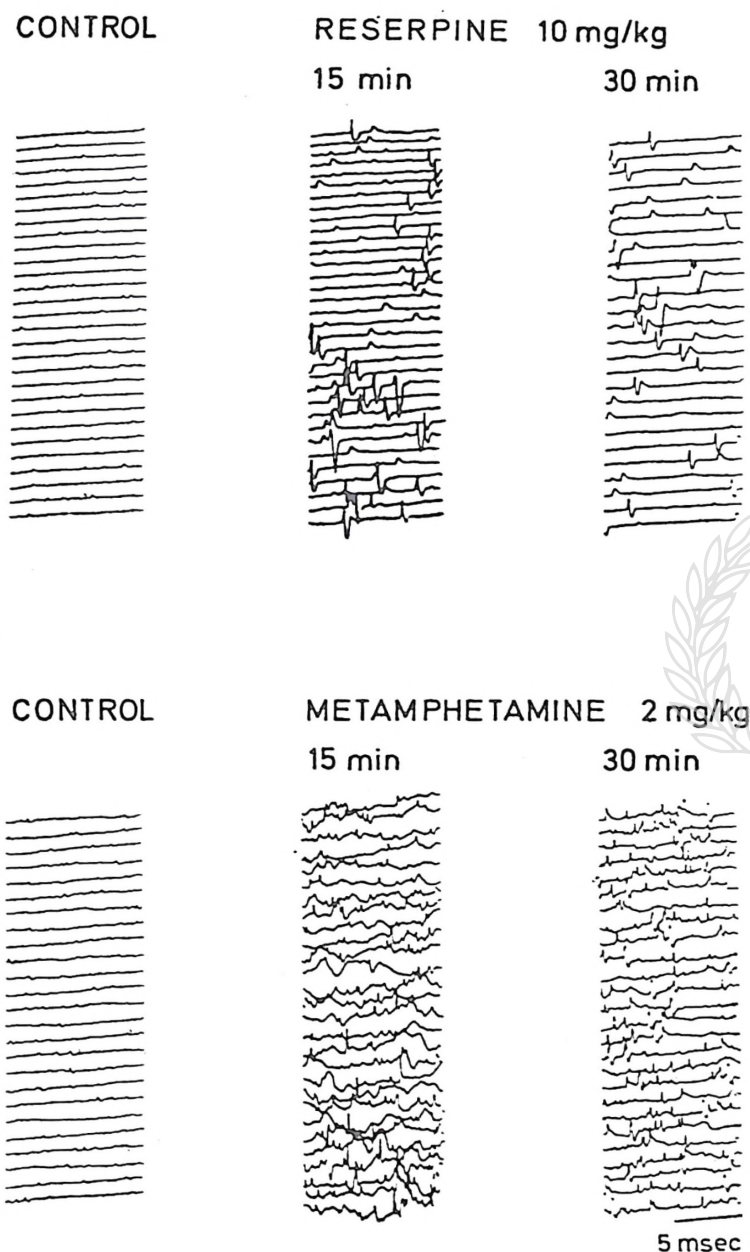


Fig. 6

Burst-like increase of spontaneous alpha discharges induced by reserpine and metamphetamine. Discharges were led off from ventral root filament. The recordings were made with running beam on running film.

such as atropine, biperiden, metixene or the adrenergic beta receptor blocking agent propranolol, abolish tremor activity, whereas drugs which inhibit exclusively alpha reflex hyperactivity, such as l-dopa depress tremor intensity but not tremor generation (Jurna et al., 1970).

The difference in the way of tremor generation by drugs which uniformly produce parkinson-like rigidity is not surprising if it is borne in mind that there are two final pathways mediating changes in spinal motor control, the alpha and the gamma system. Consequently, similar motor symptoms may be brought about by changes in activity of one or the other system, as is well illustrated by rigidity resulting from either intercollicular or anemic decerebration, which is of the gamma or the alpha type respectively. It indicates, however, that different supraspinal structures are responsible for the initiation and maintenance of parkinson-like rigidity and tremor.

With respect to the Parkinson syndrome the results may be summarized as follows (Fig. 7).

	rigidity	tremor	akinesia
reserpine	α	α	present
oxotremorine	α	γ	absent

Fig. 7

Summary of the results

Rigidity of the alpha type is produced by drugs either depressing monoaminergic or increasing cholinergic transmission, and by tremorine or oxotremorine. Tremor resulting from an application of reserpine is due to bursts in alpha motor activity, whereas oxotremorine tremor is operated by the gamma motor system. And finally, parkinson-like akinesia is only produced by drugs interfering with monoaminergic transmission, but not by oxotremorine.

I. JURNA

RIGIDITET I TREMOR MIŠA IZAZVAN LIJEKOVIMA

KRATAK SADRŽAJ

Iz dosadašnjih saznanja izvučen je zaključak da Parkinsonova bolest može rezultirati iz nedostatka ravnoteže između monoaminergičnog i holinergičkog sistema u mozgu, gdje izgleda da je deplecija dopamina u striatumu jedan od najvažnijih faktora. Bilo da smanjuju monoaminergične funkcije (kao npr. reserpin i hlorpromazin), ili povećavaju

holinergične funkcije (fizostigmin), ti lijekovi na isti način proizvode akineziju, rigiditet i tremor miša. Ovaj tip rigiditeta je nastao uslijed hiperaktivnosti alfa motornog sistema i može se prekinuti antiparkinsonskim lijekovima. Alfa-tip rigiditeta je takođe prouzrokovan tremorgeničnim agensima tremorinom i oksotremorinom, koji povećavaju koncentraciju acetilholina u mozgu.

Tremor izazvan tremorinom i oksotremorinom nastao je uslijed inhibicije aktivnosti gama motornog sistema. Gama motorni sistem ipak ne snosi odgovornost za aktiviranje tremora nakon reserpina, jer je taj alkaloid reducirao gama motorna ispražnjavanja.

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DISCUSSION

Bruggencate: 1) what are the effects of tremorine in a spinal animal? 2) there is now so much evidence accumulating for a physiological role of the so called alpha-gamma linkage (Granit, 1968; Hagbarth and Valbo, 1968) that the dissociation between alpha-gamma-activity you get, looks in a way »abnormal«. Do you think that this dissociation between alpha and gamma-activity could be related somehow to the inability of patients, suffering from rigor, to perform movements?

Jurna: 1) Tremorine had no effects on alpha or gamma motor activity in spinalized rats in the dose used. 2) There are only the results obtained on alpha and gamma reflex discharge under the influence of tremorine and oxotremorine which at first sight indicate a dissociation of alpha-gamma linkage, because of the increase in alpha reflex discharge and the unchanged or sometimes even increased gamma reflex discharge. It could well be, however, that gamma reflex discharge actually is not unchanged but reduced the reduc-

tion being masked by the increase in spontaneous gamma activity, which is always observed after application of both tremorigenic drugs. In the recordings of »reflex« alpha and gamma activity real reflex activity could not be distinguished from intermixed spontaneous activity. Depressed gamma activity, as it seen for instance in reserpine rigidity, might indeed contribute to some extent to inability to perform graded movements.

Vizi: In what concentration did you use propranolol? Propranolol has got a strong local anaesthetic effect. Thus, what do you think the effect of propranolol in abolishing tremor is exerted via beta-receptor, or it is a simple local anaesthetic action?

Jurna: Propranolol was tried in varying doses in experiments, in which only the electromyogram was recorded. The minimal dose to reduce tremor activity was 0.5 mg/kg. Suppression of tremor generation was observed after a dose of 2 mg/kg. I don't know whether in this dose the local anaesthetic action of propranolol will have to be taken into consideration. It is quite improbable, however, that the antitremor effect of the drug is due to a local anaesthetic action, because procaine does not abolish tremor generation but only depresses tremor intensity by way of reducing alpha hyperactivity.

Schnieden: I was interested in your results with propranolol. Have you tried other beta blocking agents such as practalol?

Jurna: We have also performed experiments with pronethalol and obtained similar results as with propranolol.

Bernard: It was stated by the author that propranolol depresses tremor activity after oxotremorine. Is this a central or a peripheral effect of propranolol?

Jurna: Probably it is a central effect. It is assumed that tremor activity after the administration of tremorine or oxotremorine results from gamma hyperactivity. Now alpha and gamma activity was recorded from central root filaments so that peripheral effects were eliminated.

Dimitrijević: 1) You stated there are two motor outputs alpha and gamma motor system: where you define this output in the level of ventral filaments or muscles? 2) You mentioned that oxotremorine could induce spasticity. What you call by spasticity? Please if you give your operating definition.

Jurna: 1) We identified alpha and gamma motor units in ventral root filaments. 2) The term, spasticity was used by EVERETT et al. 1956 to describe the increased muscular tone observed after application of tremorine. I have called the motor symptom consisting of an increased resistance against passive movements of feet and legs and tonic activity in the electromyogram during sustained stretch of the muscles after tremorine or oxotremorine »rigidity« because it is very similar to the respective symptom after reserpine or physostigmine. I confess, however, that I would not make the choice of one or the other term a point of serious argumentation.

György: May I ask you how does influence reserpine the effect of OT (is there a potentiation or an antagonism)?

Jurna: We have shown a reduced response to oxotremorine after pre-treatment of rats with propranolol which would fit in with your findings on the inhibition of the gamma discharges. We have also shown that reserpine will reduce oxotremorine tremor. In no case was there an abolition of the tremor only a reduction in intensity.

Stern: My experiments with preparation CIBA 28882 don't prove that rigidity is caused by activation alpha-motor system. This substance inhibits muscle spindle and gamma-cells. Fluphenazine given after mentioned preparation produces tremor rigor. After destroying alpha-cells with p-bromphenilacetilurea fluphenazine cause rigor.

DUBRAVKA POTKONJAK

THE RELATIONSHIP BETWEEN RIGIDITY AND CHANGE IN WHOLE BRAIN ACETYLCHOLINE CONCENTRATION PRODUCED BY INJECTION OF FLUPHENAZINE IN THE RAT

Arvidsson et al. (1966) advanced the hypothesis that drugs inducing a shift of the monoaminergic-cholinergic equilibrium in favour of the cholinergic neurons in the brain of experimental animals — were able to induce a state of rigidity in these animals. This rigidity manifested itself by an increased resistance against flexion of the limbs and by a reduction of gamma and an increase of alpha motoneurone response. Because of this that rigidity was said to be of an alpha type (Steg, 1964; Roos and Steg, 1964; Arvidsson et al., 1966; Jurna et al., 1969). According to Arvidsson et al. (1966 and 1967) chemically different drugs could induce the mentioned shift either facilitating cholinergic transmission (physostigmine) or inhibiting monoaminergic transmission (reserpine, chlorpromazine, phenoxybensamine, haloperidol). If the balance is restored by drugs which either stimulate monoaminergic function (L-DOPA) or depress cholinergic function (atropine) — rigidity is eliminated.

Reserpine and phenothiazine derivatives, which have been reported to produce rigidity in animals as well as in man (Glow, 1959; Courvoisier et al., 1957; Steg, 1964) — are generally believed to induce rigidity in rats by an inhibition of monoaminergic transmission (Roos and Steg, 1964; Arvidsson et al., 1966 and 1967; Hornykiewicz, 1966; Jurna et al., 1969) either depleting the stores of the transmitter substances (reserpine) or preventing the transmitter to act at the receptor site (phenothiazine derivatives). Reserpine, however, has been reported not only to inhibit monoaminergic transmission, but to affect cholinergic transmission as well. This drug has been shown to produce a significant increase in the acetylcholine content of different areas of the dog's brain (Malhotra and Pundlik, 1959), of the rat brain stem (Malpica et al., 1970) and of the whole rat brain (Giarmán and Pepeu, 1962).

The aim of this work was to investigate whether the phenothiazine derivative fluphenazine, apart from inhibiting monoaminergic transmission, can, like reserpine, affect cholinergic transmission also. In order to examine this, the relationship between rigidity and brain acetylcholine

concentration after fluphenazine administration into rats was studied. In an attempt to provide more information on this new mode of action of fluphenazine as a rigidity — producing drug — it was decided to investigate the interactions between atropine and fluphenazine and gamma-hydroxybutyrate and fluphenazine.

METHODS

Male wistar rats weighing 200 — 250 g were used in all the experiments.

Rigidity was induced by an intraperitoneal injection of 10 mg/kg of fluphenazine, which is chemically 2-trifluoromethyl-10 — {3' — [4] — (2-hydroxyethyl)-1-piperazinyl} propyl} phenothiazine. Fluphenazine induced rigidity was palpated at dorsiflexion of the rat's foot and its onset, duration and intensity were observed.

Acetylcholine was extracted from individual rat brains by the method of Mac Intosh and Perry (1950). The extracts were assayed for acetylcholine on the guinea-pig ileum pretreated with mipafox.

Rigidity and brain acetylcholine were also estimated 45 minutes after intraperitoneal administration of either 10 mg/kg of atropine sulphate or 1,5 g/kg of gamma hydroxybutyrate into rats previously pretreated with fluphenazine for 15 minutes.

Rats injected with saline were used as controls.

RESULTS

Fluphenazine induced rigidity is manifested in rats by a decreased spontaneous motor activity, by a posture of general flexion and especially by an increased resistance against dorsiflexion of the feet and toes. This resistance was weak 5 minutes after the injection of fluphenazine. After that time it increased, reached a maximum 30 minutes after fluphenazine administration and then it started to decrease. 90 minutes after fluphenazine injection this resistance was still pronounced. The time course of fluphenazine induced change in rat brain acetylcholine is shown in Fig. 1. Fluphenazine induced an increase in rat brain acetylcholine concentration. The concentration was significantly higher than the control 15 minutes after the injection of fluphenazine ($p < 0,05$). It continued to rise until 30 minutes after the injection when it reached a mean value of 5.35 $\mu\text{g/g}$ ($p < 0,001$), representing an increase over the control of about 165%. Sixty minutes after the injection of fluphenazine the brain acetylcholine concentration was still significantly higher ($p < 0,001$) than that of saline controls.

Atropine and gamma hydroxybutyrate showed in our experiments the same effect on fluphenazine induced increase in the rat brain acetylcholine concentration, but had different effects on fluphenazine induced rigidity in rats. Atropine had no measurable effect on rigidity, and gamma-hydroxybutyrate abolished it.

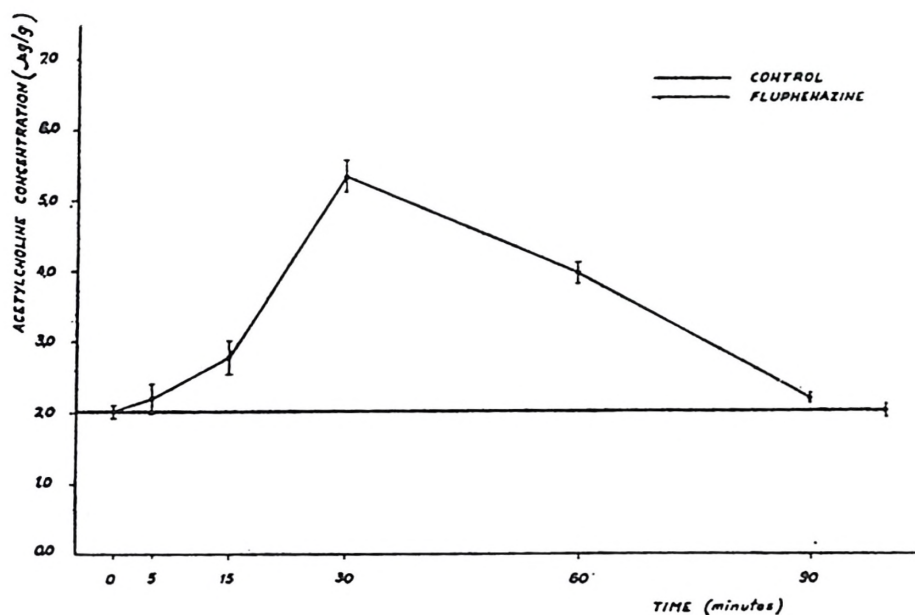


Fig. 1

The time course of fluphenazine induced change in rat brain acetylcholine concentration ($\mu\text{g/g}$ whole brain $\pm$ standard error). Ordinate: acetylcholine concentration in $\mu\text{g/g}$. Abscissa: time in minutes. The horizontal line represents acetylcholine concentration of saline pretreated controls. Each point is the mean of five determinations.

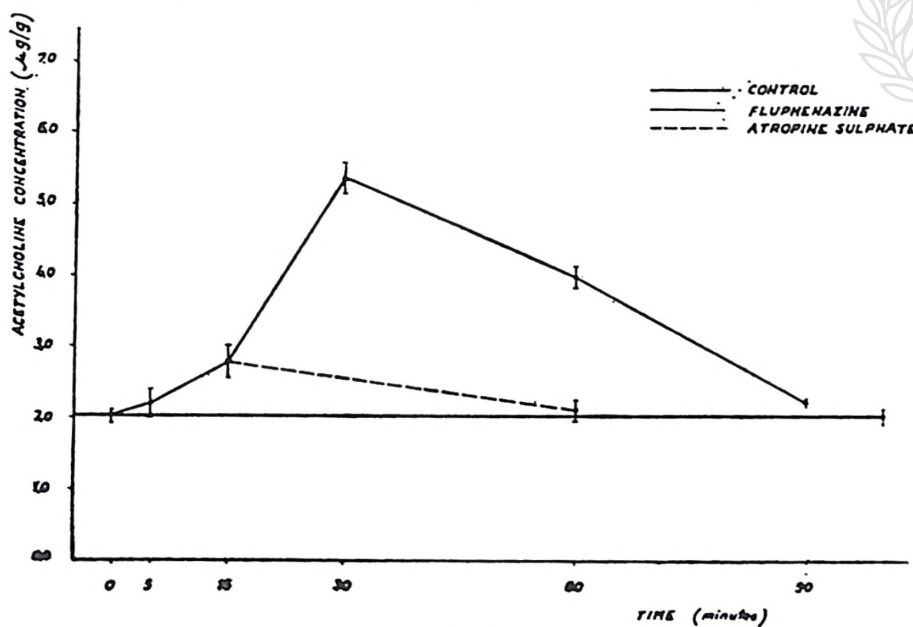


Fig. 2

Effect of atropine sulphate on fluphenazine induced increase in rat brain acetylcholine concentration ($\mu\text{g/g}$ whole brain $\pm$ standard error).

The effect of atropine sulphate on fluphenazine induced increase in rat brain acetylcholine concentration is shown in Fig. 2. The effect of gamma-hydroxybutyrate on fluphenazine induced increase in rat brain acetylcholine concentration is shown in Fig. 3. As can be seen both drugs caused a significant decrease in fluphenazine induced rise in the rat brain acetylcholine. Mean values of 2.10 $\mu\text{g/g}$ after atropine and 2.19 $\mu\text{g/g}$ after gamma-hydroxybutyrate were not significantly diffe-

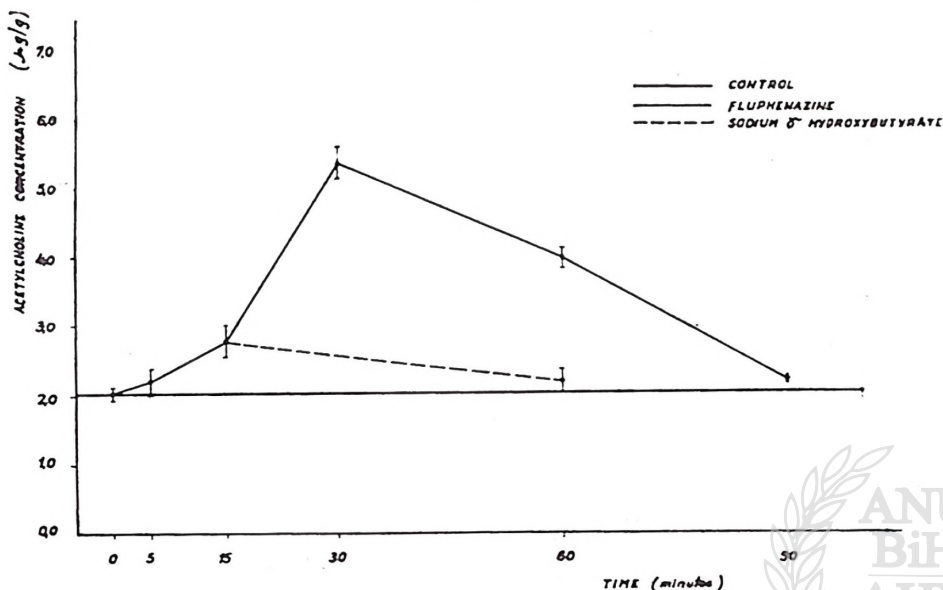


Fig. 3

Effect of gamma-hydroxybutyrate on fluphenazine induced increase in rat brain acetylcholine concentration ($\mu\text{g/g}$ whole brain $\pm$ standard error).

rent from the saline control value, but represented a significant decrease of about 45% when compared with the corresponding mean value after fluphenazine alone.

DISCUSSION

It has been generally accepted (Carlsson and Lindqvist, 1963; Roos and Steg, 1964; Hornykiewicz, 1966 and 1967; Jurna et al., 1969) that phenothiazine derivatives induce rigidity in experimental animals by an inhibition of monoaminergic transmission which is considered to be due to the blockade of catecholamines, especially at dopamine receptors in the brain. These substances thus prevent the endogenous dopamine from acting on its specific receptor and cause a functional dopamine deficiency at the synapses.

In our experiments, however, the phenothiazine derivative fluphenazine, which has been shown to be about 25 times more potent in

inducing rigidity than chlorpromazine which in this respect occupies an intermediate position among phenothiazine substances (Bohaček et al., 1963; Hornykiewicz, 1967) — produced a significant increase in rat brain acetylcholine concentration during the state of rigidity. The onset, intensity and duration of rigidity and the rise in brain acetylcholine concentration coincided with each other. This could mean that fluphenazine induced rigidity and the increase in brain acetylcholine were causally related and that fluphenazine might be able to produce rigidity by causing an imbalance between monoaminergic and cholinergic systems in the brain in two ways: by inhibiting monoaminergic transmission as well as by facilitating cholinergic transmission. At present little work has been reported concerning the effect of phenothiazines on acetylcholine concentration. There have been some reports that chlorpromazine reduced the activity of acetylcholine esterase (Kivalo et al., 1958). Bošković (1970) investigated the effect of fluphenazine on the acetylcholine esterase activity both in vitro and in vivo and he found that this substance caused a significant decrease in activity of acetylcholine esterase in vitro. He was unable, however, to detect any significant effect of fluphenazine on that enzyme in vivo by means of histochemical methods used. Thus the mechanism of fluphenazine induced rise in brain acetylcholine concentration in vivo is at present still uncertain and is yet to be explained. The results obtained with atropine and gamma-hydroxybutyrate seem to indicate that fluphenazine, might produce rigidity by causing an imbalance between monoaminergic and cholinergic functions in the brain in two ways: by inhibiting monoaminergic transmission as well as by facilitating cholinergic transmission.

Atropine has been reported to restore a drug induced shift of the monoaminergic-cholinergic equilibrium and abolish rigidity by depressing cholinergic functions (Arvidsson et al., 1966, Jurna et al. 1969). However, succeeded to eliminate reserpine induced rigidity (which has been reported to be of the same type as phenothiazine rigidity) by pretreatment with atropine, but found it difficult to demonstrate an antagonising effect when atropine was applied after reserpine induced rigidity had fully developed. In our experiments atropine was administered into rats which already exhibited fluphenazine induced rigidity and was not able to abolish it, but did produce a decrease in brain acetylcholine. The only possible explanation for these effects of atropine might be the already mentioned two ways fluphenazine induced shift in the monoaminergic-cholinergic equilibrium in the brain. Rigidity which persisted after atropine administration was probably due to the fluphenazine induced inhibition of monoaminergic transmission and atropine has not been shown to inhibit the monoaminergic transmission.

Results obtained with gamma-hydroxybutyrate give more support to the opinion that fluphenazine might produce rigidity by causing an imbalance between monoaminergic and cholinergic functions in the brain in two ways. Gamma-hydroxybutyrate has been reported to produce a selective, dose-dependent increase of brain dopamine in mammals (Gessa et al., 1966) and in our experiments it abolished induced rigidity and produced a decrease in brain acetylcholine, the mecha-

nism of which is still to be explained. Causing a selective increase of brain dopamine and a significant decrease in brain acetylcholine this drug restored the shift of monoaminergic-cholinergic equilibrium induced by fluphenazine — and abolished rigidity.

D. POTKONJAK

ODNOS IZMEĐU RIGIDITETA I PROMENA U KONCENTRACIJI UKUPNOG ACETILHOLINA IZ CELOG MOZGA ŠTAKORA IZAZVANIH POMOĆU FLUFENAZINA

KRATAK SADRŽAJ

Objavljeno je da supstance koje izazivaju pomeranje ravnoteže između monoaminergičnog i holinergičnog sistema u korist holinergičnih neurona (bilo facilitacijom holinergičnih ili inhibicijom monoaminergičnih neurona) u mozgu eksperimentalnih životinja (štakora) — mogu dovesti do pojave rigiditeta kod tih životinja.

Intraperitonealna primena flufenazina izazvala je rigiditet kod štakora. U isto vreme koncentracija ukupnog acetilholina iz celog mozga tih štakora bila je signifikantno povišena.

Atropin sulfat nije imao primetnog uticaja na rigiditet izazvan flufenazinom, ali je izazvao signifikantno sniženje koncentracije ukupnog acetilholina iz celog mozga štakora.

Gama-hidroksibutirat je potpuno uklonio rigiditet izazvan pomoću flufenazina i doveo je do signifikantnog sniženja koncentracije ukupnog acetilholina iz celog mozga tretiranih životinja.

Neki od ovih rezultata izgleda da upućuju na to da flufenazin može izazvati rigiditet prvenstveno pomoću facilitacije holinergičnog sistema, a u manjoj meri pomoću inhibicije monoaminergičnih neurona do koje dolazi zbog blokade dopaminskih receptora, što dovodi do funkcionalnog deficita dopamina u sinapsama mozga štakora.

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ELVEDINA ZVIZDIĆ AND SEID HUKOVIĆ

NEUROMUSCULAR EFFECT OF TREMOGENIC AGENTS IN VITRO*

INTRODUCTION

The site of action of oxotremorine as a cause of many features of Parkinson's disease is in central nervous system (5), in particular in the rat in the midbrain reticular formation (8, 16). Tremorine tremorgenesis is the result of excitation of a pacemaker in the tegmental reticular formation at the mesencephalic-pontine junction (17). At the same time evidence of peripheral involvement has been demonstrated (3, 4, 7). Other substances used as pharmacological tools for the evaluation of antiparkinson drugs (6) as nicotine (1), harmine (20) and physostigmine (15) act in the central and peripheral nervous system also.

Since little quantitative informations are available concerning the intensity of the effect of oxotremorine, harmine, nicotine and cyclopium (19), on neuromuscular transmission in comparison to its effect in central nervous system, it was investigated the influence of tremorgenic agents on the effect of nerve stimulation of isolated vas deferens, urinary bladder and oesophagus of rats and mice. The first preparation is used as a model-system for adrenergic nerve smooth muscle transmission (11), isolated urinary bladder is used as a cholinergic nerve smooth muscle preparation (12) and the third model-system is used for striated muscle and cholinergic nerve transmission (14).

METHOD

The animals used were rats and mice. The rats weighted 200 g and mice 25 g. The animals were stunned by a blow on the head and then decapitated. The abdomen and the thorax were opened along the middle line and ductus deferens and urinary bladder were exposed. The hypogastric adrenergic nerve was tied with small piece of peritoneum half cm from ductus deferens. The pelvic nerve was tied around ureter for the pelvic nerve run close to the ureter. The heart and lungs were removed to

* This work was supported by Federal Grant of Yugoslavia, Beograd.

expose the oesophagus. The longitudinal pair of *rami oesophagei nervi vagi* were tied as near as possible to the oral part the thorax. The organs were put in Tyrode solution first and then were set up in the isolated organ bath. One day oesophagus was set up first, second day urinary bladder was first and third day ductus deferens was first organ that was set up as fresh as possible. The organ bath contained 40 ml of Tyrode solution that was warmed to $32^{\circ}\text{C} \pm 0,5$ and bubbled with 95% O_2 and 5% CO_2 . The proximal part of ductus deferens was connected with frontal lever. The fundus of urinary bladder was connected with frontal lever too. Frontal lever was connected with proximal part of ductus deferens cardinal part of oesophagus and fundus of urinary bladder. The magnification being 4 and the load for the rats organs one gram and for the mice organs half gram. The tied nerve were put through bipolar electrodes as was described (2). Submaximal strength of electrical stimuli, 1 msec. duration at a frequency of 40 Hz were applied every min. or half min. for 2 sec.

Ten control contractions were recorded and the slow infusion of 0,02 ml/min in 40 ml organ bath started. Substances dissolved in Tyrode solution were infused: Oxotremorine 10^{-2} M, Harmine 10^{-3} M, Nicotine 10^{-3} M and Cyclopium 10^{-3} M/ml. One organ one substance. Three concentrations were found and expressed as a negative logarithm of molar concentration: pSm, pB75 and pB50, they are so called pharmacological constants (18). The first point is pSm, the place on base line where a molar concentration induced the highest contraction. The second point calculated is pB75, concentration that induced 75 percent of control contraction. The third point is pB50 molar concentration of drugs that reduced hight of contraction to the half of control one. As greater pharmacologic constant as greater chemosensitivity.

RESULTS

A. The effect of electrical stimulation

The effect of electrical stimulation of nerves is contraction. The responses can be repeated if the frequency of stimulation was not often and less then 2 in min.

a. *Oesophagus of rats and mice.* The effect of electric nerve stimulation is rapid contraction. The aequal reactions can be repeated for more than two hours induced by constant stimuli.

b. *Urinary bladder of rats and mice.* The effect of stimulation of control organs is slow and uniform contraction if the constant stimulus were applied.

c. *Ductus (Vas) deferens of rat.* The effect of constant stimulation of adrenergic hypogastric nerv is constant contraction, that can be repeated more than an hour if the frequency of the stimulation by the trains of stimuli is not more then 2/min.

B. The effect of tremogenic agents

All substances were infused by infusion pump 0,5 μ l/ml/min. The bath was washed out thoroughly after termination of infusion and the record of the control contraction was again taken. The organs taken from mice are more sensitive than from rats.

a. *Oxotremorine*. It induced first increase of the nerve stimulation followed by its decrease and block of the nerve conductance. The organs taken from mice are more sensitive toward oxotremorine then organs taken from rats. The most sensitive is oesophagus of mouse and the least sensitive is the effect of adrenergic nerve stimulation on ductus deferens of rat. The differens between maximal excitatory effect

Tab. 1.

THE INFLUENCE OF THE SLOW INFUSION OF OXOTREMORINE
5 x 10⁻⁶ M/min./ml ON THE EFFECT OF NERVE STIMULATION
OF ISOLATED ORGANS

Organs		pSm*	pB75	pB50
Oesophagus	rat	3,65 $\pm$ 0,25	3,20 $\pm$ 0,16	2,92 $\pm$ 0,57
	mouse	4,00 $\pm$ 0,01	3,67 $\pm$ 0,12	3,66 $\pm$ 0,11
Urinary bladder	rat	3,60 $\pm$ 0,13	3,11 $\pm$ 0,18	— (0/6)**
	mouse	3,66 $\pm$ 0,27	3,47 $\pm$ 0,19	— (0/6)
Ductus defferens	rat	3,23 $\pm$ 0,03	2,82 $\pm$ 0,17	— (0/6)
	mouse	3,72 $\pm$ 0,15	3,15 $\pm$ 0,18	— (0/6)

* pS and pB are negative logarithm of the molar concentration of oxotremorine that induce increase (S) or inhibition (B) of the effect of nerve stimulation.

** Numbers in brackets are frequency of recorded effect in relate to number of experiments.

and considerable inhibitory effect (pB75—pS), is less than one range of value. The greater degree of inhibition was not achived in smooth muscle organs (Tab. 1).

b. *Harmine* induces biphasic effect of all organs used. Exceptions are rat's oesophagus and mouse's urinary bladder. The difference at concentration between maximal effect and considerable decrease is not great as not so difference is not great between other constants (pSm — pB₇₅, pB₇₅ — pB₅₀) (Tab. 2 and Fig. 2).

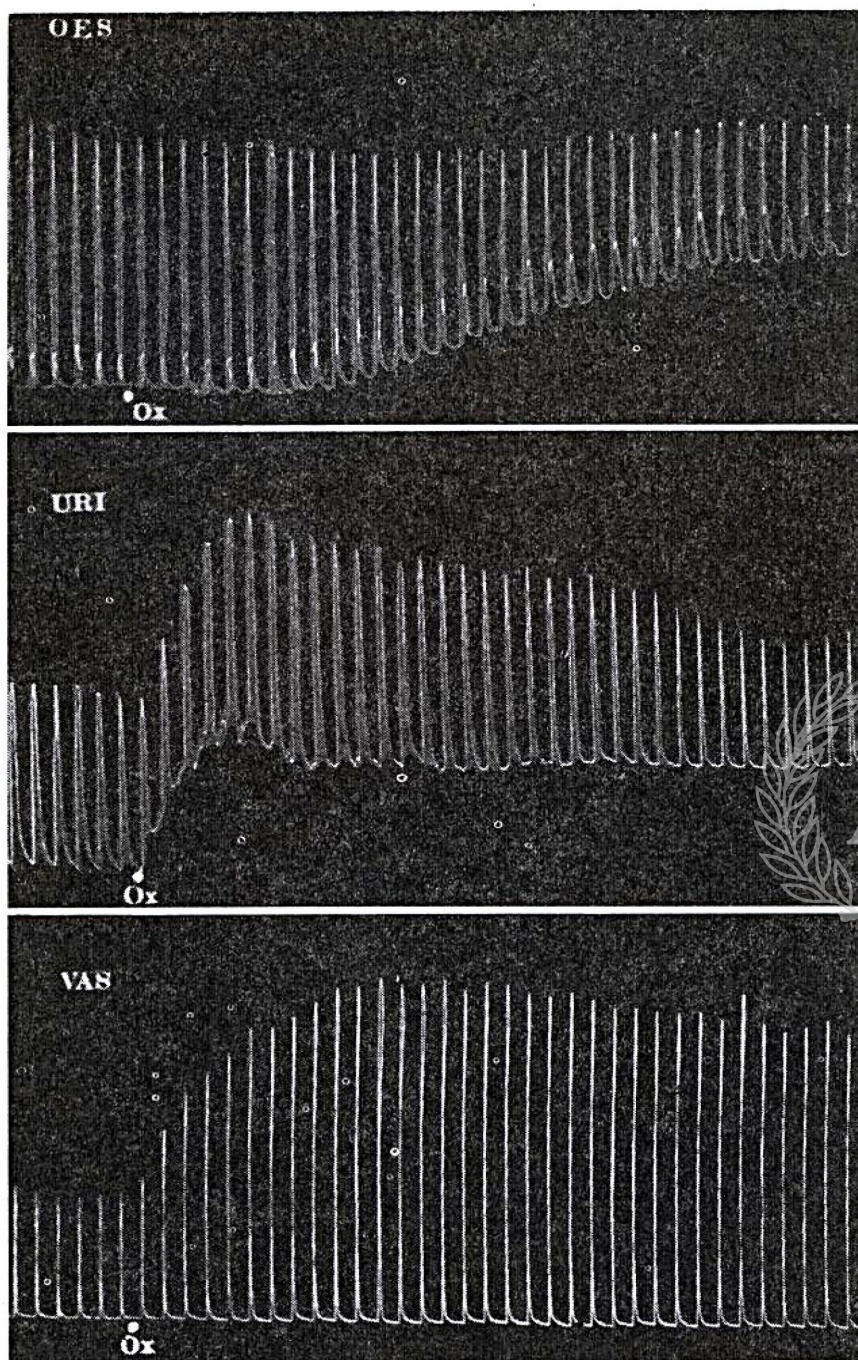


Fig. 1

The contractions of rat's isolated organs induced by the electrical nerve stimulation (5V, 1mSec, 40 Hz, every min. for 2 Sec.). At the point Ox infusion of oxotremorine 5×10^{-6} M/ml/min stated. Oes is oesophagus, Uri is urinary bladder, vas is ductus deferens.

Tab. 2.

THE INFLUENCE OF THE SLOW INFUSION OF HARMINE
 5×10^{-7} M/min./ml ON THE EFFECT OF NERVE STIMULATION
 OF ISOLATED ORGANS

Organs		pSm*	pB75	pB50	pB25
Oesophagus	rat	— (0/6)**	$5,03 \pm 0,24$	$4,91 \pm 0,34$	$4,59 \pm 0,16$
	mouse	$5,53 \pm 0,18$	$5,26 \pm 0,18$	$5,12 \pm 0,15$	$4,96 \pm 0,15$
Urinary bladder	rat	$5,43 \pm 0,13$	$4,73 \pm 0,71$	$4,61 \pm 0,89$	$4,49 \pm 0,09$
	mouse	— (0/6)	$5,68 \pm 0,55$	$5,03 \pm 0,21$	$4,83 \pm 0,99$
Ductus deferens of rat		$6,20 \pm 0,98$	$5,60 \pm 0,13$	$5,80 \pm 0,01$	$4,84 \pm 0,33$

* pS and pB are negative logarithm of the molar concentration of harmine that induce increase (S) or inhibition (B) of the effect of nerve stimulation.

** Numbers in brackets are frequency of recorded effect in relate to number of experiments.

c. *Nicotine*. Continual slow infusion of nicotine $10^{-6,3}$ M/ml/min. does not change significantly the effect of nerve stimulation. The effect of nicotine is biphasic on the smooth muscle organs with adrenergic nerve stimulation. The first effect is the increase of stimulation on the vas deferens, pS_m is 5,77 then follows decrease pB₇₅ is 5,25 and pB₅₀ is 4,88. The difference is 0,5 range of value. The striated muscle of oesophagus responded up on the simultaneous application of drug and electrical stimulation with the smaller contraction then upon the electric stimulation of the nerve only (Tab. 3).

Tab. 3.

THE INFLUENCE OF THE SLOW INFUSION OF NICOTINE
 5×10^{-7} M/min./ml ON THE EFFECT OF NERVE STIMULATION
 OF ISOLATED ORGANS

Organs		pSm*	pB75	pB50
Oesophagus	rat	5,78 (1/6)**	5,64 (1/6)	5,56 (1/6)
	mouse	5,77 (1/6)	$5,25 \pm 0,87$	$5,14 \pm 0,63$
Urinary bladder	rat	6,10 (1/6)	$5,34 \pm 0,17$	$5,16 \pm 0,14$
	mouse	— (1/6)	$4,89 \pm 0,54$	— (0/6)
Ductus deferens of rat		$5,77 \pm 0,17$	$5,25 \pm 0,13$	$4,88 \pm 0,55$

* pS and pB are negative logarithm of the molar concentration of nicotine that induce increase (S) or inhibition (B) of the effect of nerve stimulation.

** Numbres in brackets are frequency of recorded effect in relate to number of experiments.

d. *Cyclopium*. Slow infusion of cyclopium $10^{-6,3}$ M/ml/min. induced gradual increase of the effect of stimulation. The maximal effect was recorded in concentration $10^{-5,5}$ M. The increased effect was seen in all six preparations. (Fig. 3).

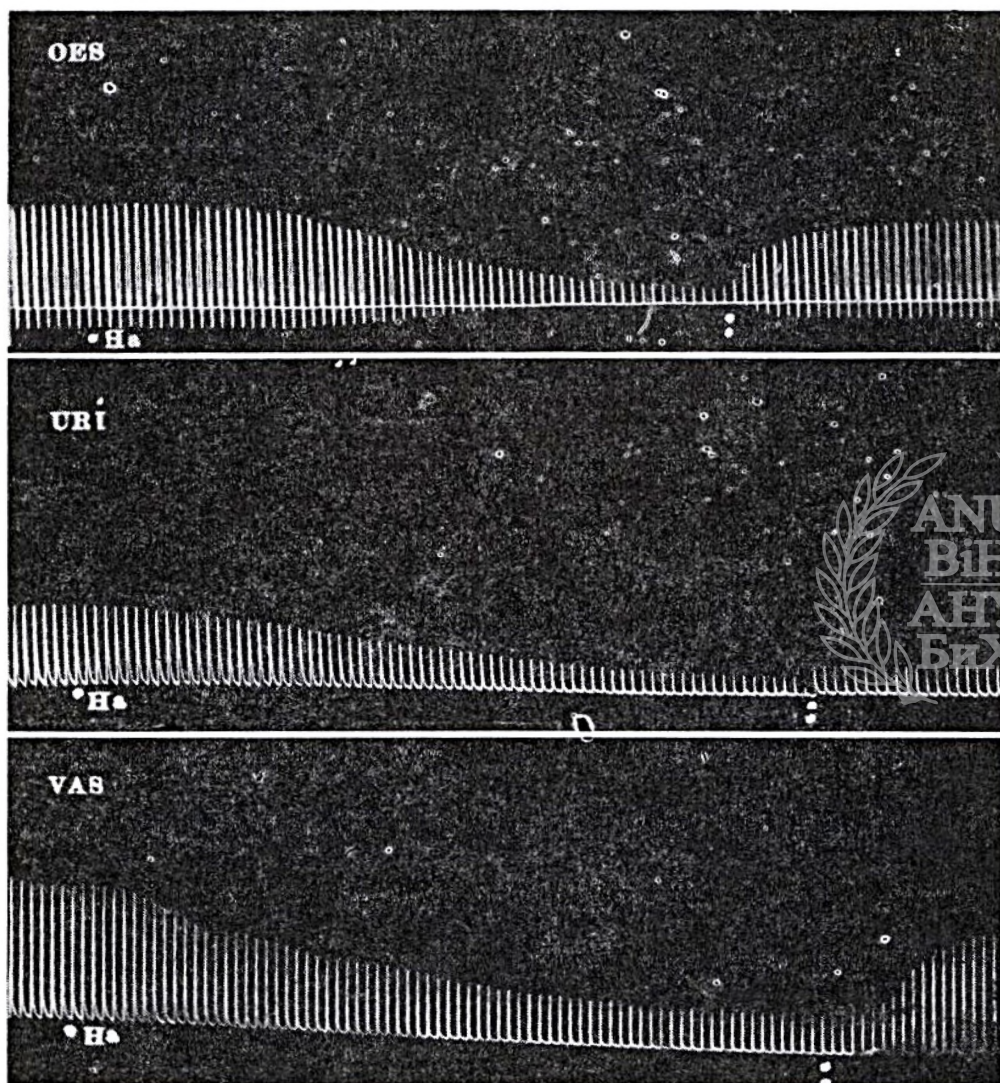


Fig. 2

The contractions of mouse isolated organs induced by the electrical stimulation (5 V, 1 mSec., 40 Hz, every 30 Sec for 2 Sec). At the point Ha infusion of harmine 5×10^{-7} M/ml/min. Oes is oesophagus, Uri is urinary bladder, Vas is ductus deferens.

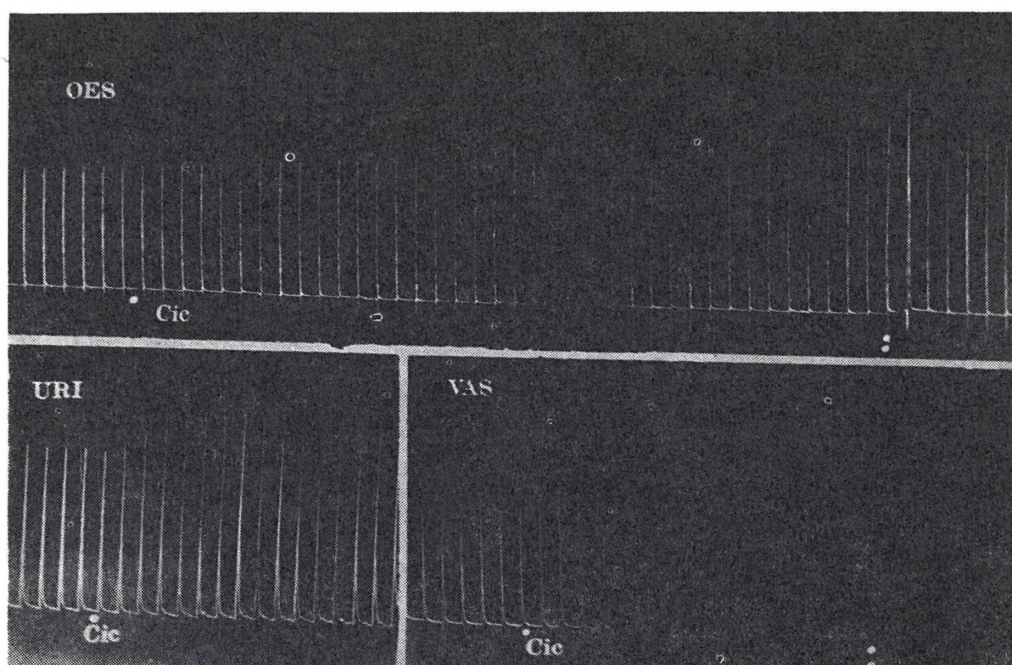


Fig. 3

The contractions of rat's isolated organs induced by the electrical nerve stimulation (5 V, 1 mSec, 40 Hz, every min. for 2 Sec). At the point CIC infusion of cyclopiam $10^{-6.3}$ M/ml/min. Oes is oesophagus, Uri is urinary bladder, Vas is ductus deferens.

DISCUSSION

Oxotremorine, harmine and cyclopiam increase the effect of nerve stimulation of great part of preparations used in this work. The excitatory effect of oxotremorine was followed by decrease of the height of contractions. The difference $pB_{75} - pS_m$ was of low range of value 0.5 approximately. Harmine induced biphasic reactions, exceptions are rat's oesophagus and mouse's urinary bladder. Nicotine was not effective on rat's oesophagus. Its stimulating effect is not frequent except on ductus deference.

The effect of oxotremorine at the indirectly stimulated junction was reported by Ganguly and Chaudhury (8). Their results may explain oxotremorine induced tremor and rigidity involving its effect on periphery. Oxotremorine induced excitatory effect by releasing of acetylcholine (13), or by depolarisation at the muscle endplate (7). It is suggested that oxotremorine does not act *per se* but by mobilization of acetylcholine (10). Oxotremorine is substance with peripheral muscarinic properties which penetrate into the central nervous system and produces tremor in mice and rats (9). It has no nicotinic action but might be producing its effects (18) by increasing activity in the efferent sympathetic

nervous system. The peripheral effect of oxotremorine seen in this work could be explained by mobilizing effect on acetylcholine in the cholinergic and adrenergic nerves. Cyclopium induced smooth muscle contraction (19).

The concentration of oxotremorine used *in vitro* and expressed as negative logarithm are of the 4 range of value and that used *in vivo* for tremor are 5 and more. The difference is 1 to 2 range of value. It means that the central site of action is predominant and if periphery was involved the excitatory effect would be the most important. The same could be said for cyclopium, *in vivo* produce the effect in the range of value 6 or more. *In vitro* only at 5. Harmine and nicotine induced tremor at about 4,5 or 5,0 and was effective *in vitro* at 5,5 or 6,0 range of value. The difference was more than one range of value on account periphery that might suggest prevalent involvement of peripheral nervous system and muscle in tremorgenezis. The first significant effect of nicotine and harmine was depression of the nerve stimulation on striated muscle and their tremorgenic effect might be connected with depression and not excitation.

SUMMARY

Oxotremorine, harmine, nicotine and cyclopium exerts an action on the contraction induced *in vitro* by nerve stimulation of oesophagus, urinary bladder and ductus deferens of rats and mice. For different extent of the effect of drugs the dimensions were expressed as negative logarithm of molar concentration pS_m , pB_{75} and pB_{50} .

Oxotremorine and harmine induced biphasic reaction. Increase of contraction of muscle followed by decrease of the effect of nerve stimulation. The nerves were cholinergic and adrenergic and muscle were striated and smooth ones. Nicotine decreased the effect of cholinergic nerve stimulation on both kind of muscle but increased the effect of adrenergic nerve stimulation.

Comparing the concentration of oxotremorine for inducing the effect *in vitro* with the same *in vivo* it could be seen that the difference is one range of value in favour of *in vivo* effect. It is opposit for harmine and nicotine. The conclusion was drawn that the site of tremogenic effect of oxotremorine is in central nervous system but the site of action of harmine and nicotine should be in periphery. If the peripheral effect of oxotremorine and cyclopium contributed to the tremorgenezis it contributed by excitatory effect, vice versa, if harmine and nicotine contributed to tremorgenezis they contribute with their depressive effect.

**UTICAJ TREMOGENIH SUPSTANCI NA NEUROMIŠIĆNU
TRANSMISIJU IN VITRO**

KRATAK SADRŽAJ

Oksotremorin i druge tremogene supstance imaju mjesto djelovanja u sinapsama centralnog nervnog sistema, a posebno u mezencefaličkoj retikularnoj formaciji. Osim ovog mjesta djelovanja, poznato je da tremogene supstance mogu djelovati na periferiji, pa je cilj ovog rada da se dobiju kvantitativni podaci o djelovanju na neuromišićnoj transmisiji. Upotrijebljena su tri tipa nerava i dva tipa muskulature. Uzeti su autonomni adrenergični i holinergični nervi i somatski holinergični nerv, te glatka i poprečnoprugasta muskulatura. Izazvane su kontrakcije mišićnih organa in vitro submaksimalnom električnom stimulacijom nerava i aplicirane tremogene supstance.

Izolirani su ezofagus, mokraćni mjehur i duktus deferens štakora i miša sa pripadajućim nervima. Poslije adaptacije i kontrolnih kontrakcija infundirani su oksotremorin, harmin, nikotin i ciklopijum tačno određene koncentracije i brzine. Efekt pomenutih supstanci je bio izražen farmakološkim konstantama pS_m , pB_{75} i pB_{50} . Oksotremorin, harmin i ciklopijum izazivaju bifazičku reakciju, tj. prvo povećanje, iza čega uslijedi smanjenje efekta stimulacije nerava. Razlika između pB_{75} do pS_m je malog reda veličina oko 0,5. Nikotin je bez efekta na ezofagusu štakora, a stimulira samo efekt adrenergične nervne stimulacije na duktus deferensu. Koncentracija oksotremorina upotrijebljena u ovom radu in vitro u uporedbi sa efektivnom dozom in vivo veća je za jedan i više redova veličina, što znači da je centralno mjesto djelovanja u CNS-u predominantno. Obratno, manje je supstance potrebno in vitro za vidljiv efekt harmina i nikotina, što ukazuje da oni djeluju na periferiji. Ako oksotremorin i ciklopijum doprinose tremogenezi, onda djeluju ekscitirajući, vice versa, ako harmin i nikotin doprinose tremogenezi, doprinose depresivnim efektom.

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DISCUSSION

Bošković: 1) How one can explain the inhibitory effect of oxotremorine on the striated muscle? 2) Does Harmine, under certain experimental conditions has an atropine-like action.

Huković: 2) H depends on experimental conditions. The explanation of the effect of Harmine, which induced decreased sensitivity toward transmitters, is not contradictory with explanation of the effect of atropine. Harmine and atropine are unexpected kinds of antagonist sometimes, because Harmine decreased and Atropine increased the effect of oxotremorine could be of three kinds dependent on species and isolated organs. I think that the effect could be direct on receptor, indirect through the nerve and inhibition of cholinesterase, the resultant of these mechanism induced reaction.

M. RADIVOJEVIĆ AND S. HUKOVIĆ

**THE EFFECT OF OXOTREMORINE AND HARMINE INJECTED
IN VIVO ON THE SENSITIVITY TOWARDS ACETYLCHOLINE,
NORADRENALINE AND ATROPINE IN VITRO***

INTRODUCTION

Oxotremorine acts as a powerfully cholinergic drug inducing tremor, rigidity, bradykinesia and alteration brainstem monoamines, all symptoms reminiscent of Parkinsonism (3, 12, 13). Tremorine tremorigenesis is a central phenomenon and is the result of excitation of a pacemaker in the tegmental reticular formation at the mesencephalic-pontine junction (14). Harmaline induces in rats and monkeys tremor similar to that of tremorine, bilaterality, sensory inhibition and independence from corticospinal pathways (14, 11). Harmine besides occasionally induces rigidity and frequently convulsions in a variety of species including primates (5).

The two drugs oxotremorine and harmine induce potent peripheral effect at the neuromuscular junction, particularly oxotremorine (4, 6). The evidence of peripheral involvement has been demonstrated in genesis of tremor and rigidity produced by oxotremorine (10). Oxotremorine potentiated acetylcholine induced contractures of chronically denervated rat hemidiaphragm *in vitro*.

The results may be explained by mechanism of receptor desensitisation (4).

Katz and Miledi have shown that hypersensitivity occurred after muscle injury, no matter whether the nerve was present or not (10). These results pointed to the importance of the presence of atrophying or degenerating muscle fibres for the development of hypersensitivity and also suggest that an increase of chemosensitivity can be expected when the muscle fibre is little damaged (15). The hypersensitivity can be greatly reduced within a few hours only by activity of the muscle fibres (12).

Seven days one after another injecting of oxotremorine and harmine to rats and mice induce lasting tremor and changed muscle activity. This activity could be induced by altered chemosensitivity at the muscle

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fibre or at the end-plate. Tremogenic agents could induce hyperactivity or muscle injury and change activity in two directions. The aim of this work is to record sensitivity of different neuromuscular junctions towards acetylcholine, noradrenaline and atropine taken from control animals and animals previously treated seven days with oxotremotone and harmine. The isolated organs used were smooth muscle and striated muscle organs of rats and mice. Two of them are with cholinergic nerve transmission oesophagus and urinary bladder, former with striated muscle and later with smooth muscle. One is ductus deferens (vas deferens) smooth muscle with adrenergic hypogastric nerve.

METHOD

It was used three groups of male rats and male mice. The first two groups of rats and mice were injected i. p. 0,25 mg/kg of oxotremotone. The second two groups of animals were injected 20 mg/kg of harmine i. p. and third two groups were injected with saline. The animals are stunned by a blow on the head and then decapitated. The abdomen and thorax were opened along the midline and ductus deferens and urinary bladder was exposed. The hypogastric nerve was tied with small piece of peritoneum 1/2 cm from ductus deferens (7). The pelvic nerve was tied around ureter for the pelvic nerves to the bladder run close to the ureter (8). Ductus deferens was prepared only of rats. The heart and lungs were removed to expose the oesophagus. The longitudinal pair of *rami oesophagei nervi vagi* were tied as near as possible to the cranial part of thorax (9). The organs were set up in the isolated organ bath containing 40 ml of Tyrode solution. The solution was warmed to $32 \pm 0,5$ and bubbled with 95% O_2 and 5% CO_2 . The proximal part of ductus deferens and fundus of urinary bladder were tied to frontal lever. The magnification being 4 and the load for the rats organs one gram and for the mice organs 0,5 g. The tied nerves were put through bipolar electrodes as was described^(1,16). Submaximal strength of electrical stimuli 1 msec. duration at a frequency of 40 Hz were applied every min. for 2 sec.

After adaptation period of 10 minutes and record of few control contraction slow infusion of 0,02 ml/min 10^{-4} M of drugs started. The infusion lasted an hour. The drug induced were acetylcholine chloride, noradrenaline chloride and atropine sulphate. Three points were found: pS_m , pB_{75} and pB_{50} , so called pharmacological constants. The first point is pS_m , the place on base line where a molar concentration induced the highest contraction. The second point is pB_{75} , concentration that induced only 75 percent of control contraction. The third point is pB_{50} molar concentration of drugs that reduced height of contraction to the half of the control one. The prefix p means negative logarithm of the molar concentrations. As greater pharmacologic constant as greater chemosensitivity.

RESULTS

A. The effect of electrical stimulation

Electrical stimulation of nerves with trains of pulses caused contraction of oesophagus, urinary bladder and ductus deferens. Constant stimuli produced constant height of contractions in a single preparation. There were not qualitative difference of effect of nerve stimulation between control and test preparation taken from animals previously treated with oxotremorine and harmine.

B. The effect of drugs on responses to electrical stimulation.

1. Infusion of acetylcholine

Acetylcholine caused biphasic response i. e. the increase of the effect of nerve stimulation followed by decrease, depression and block. The organs taken from mice are more sensitive then organs taken from rats. There is no great difference in concentration for increase and decrease of the effect of stimulation, it is less then 5 times molar concentration or less then 0,7 range of value.

Response of the oesophagus taken from the mice pretreated with oxotremorine is less sensitive towards acetylcholine then control organs. The remaining organs with cholinergic nervous supply are more sensitive. The response of ductus deferens to stimulation is increased by acetylcholine in animals treated with oxotremorine (Fig. 1). Harmine pretreatment decreased the sensitivity toward acetylcholine in all three preparations (Tab. 1).

Tab. 1.

THE INFLUENCE OF SLOW INFUSION OF ACETYLCHOLINE
(5×10^{-7} M/ml/min.) ON THE RESPONSE OF ISOLATED
ORGANS INDUCED BY ELECTRICAL NERVE STIMULATION

Organ	Animal		Seven days injections i. p.		
			Saline	Oxotremorine	Harmine
Oesophagus		pSm	5,68 (6/8)	5,68 (1/6)	5,61 (2/6)
				5,35 (6/6)	— (0/6)
		pB50	4,97 (3/8)	4,98 (5/6)	— (0/6)
	Mouse	pSm	5,88 (3/6)	4,94 (3/6)	—
		pB75	5,05 (5/6)	4,55 (5/6)	—
		pB50	5,10 (1/6)	4,62 (2/6)	—
Urinary bladder	Rat	pSm	5,34 (5/9)	5,35 (2/6)	— (0/6)
		pB75	5,21 (4/9)	5,32 (4/6)	5,19 (4/6)
		pB50	— (0/9)	4,72 (2/6)	5,07 (1/6)
	Mouse	pSm	5,57 (5/9)	— (0/6)	—
		pB75	4,90 (6/9)	5,75 (6/6)	—
		pB50	4,87 (2/9)	5,42 (2/6)	—
Ductus deferens	Rat	pSm	5,19 (9/9)	5,60 (4/7)	5,11 (6/6)
		pB75	4,88 (3/9)	5,55 (3/6)	— (0/6)
		pB50	4,80 (2/9)	4,81 (3/6)	— (0/6)

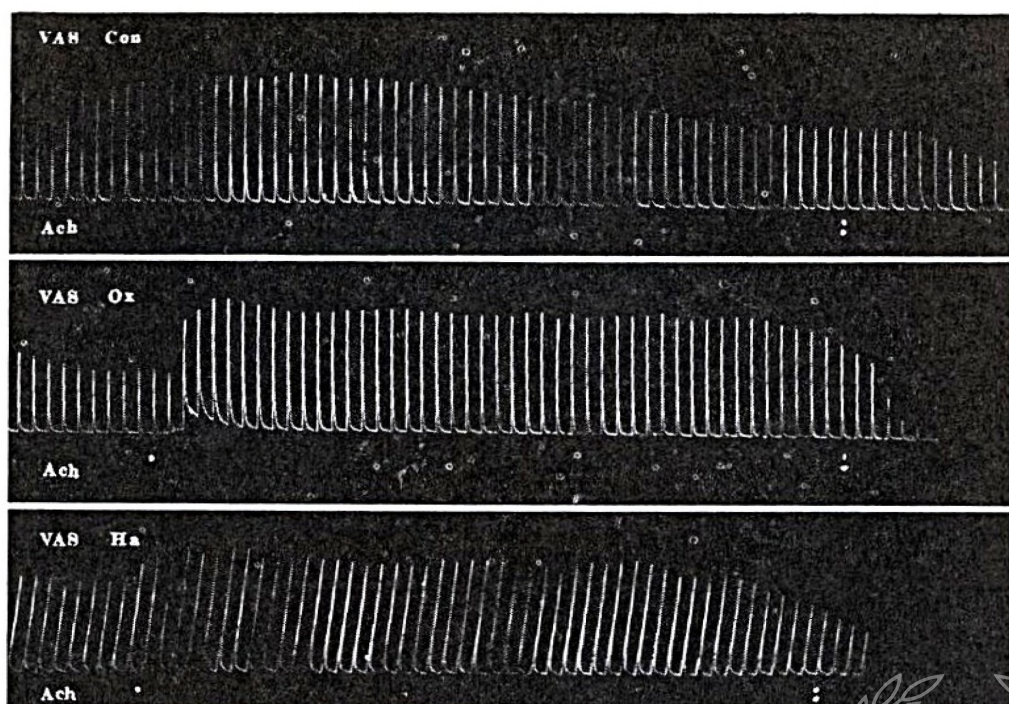


Fig. 1

The contractions of the vas deferens produced the electrical hypogastric nerve stimulation. (5V, 1 mSec, 4 Hz every min. for 2 sec.) At the point acetylcholine infusion started 10^{-7} M/ml/min. The organs were taken from (con) controle (Ox), oxotremorine and (Ha) harmine treated animals.

2. Infusion of noradrenaline

Noradrenaline induced biphasic response on ductus deferens. Increase of the effect of nerve stimulation is followed by decrease. The difference in concentration is 0,7 range of value. The same result was recorded on mouse oesophagus. The effect of noradrenaline on other controle organs was decrease of the effect of stimulation.

Tab. 2.

THE INFLUENCE OF SLOW INFUSION OF NORADRENALINE (5×10^{-7} M/ml/min.) ON THE RESPONSE OF ISOLATED ORGANS INDUCED BY ELECTRICAL NERVE STIMULATION

Organ	Animal		Seven days injections i. p.		
			Saline	Oxotremorine	Harmine
Oesophagus	Rat	pSm	— (0/6)	5,03 (3/6)	— (0/6)
		pB75	4,95 (4/6)	4,94 (1/6)	4,47 (2/6)
		pB75	4,52 (1/6)	— (0/6)	— (0/6)
	Mouse	pSm	5,31 (5/6)	5,50 (6/7)	—
		pB75	5,36 (2/6)	4,94 (1/7)	—
		pB50	4,88 (2/6)	4,91 (1/7)	—

Urinary bladder	Rat	pSm	— (0/6)	— (0/6)	5,46 (2/6)
		pB75	5,07 (6/6)	5,31 (3/6)	4,85 (4/6)
		pB50	4,97 (3/6)	5,52 (2/6)	— (0/6)
	Mouse	pSm	— (0/10)	— (0/6)	—
		pB75	5,59 (10/10)	5,88 (4/6)	—
		pB50	5,43 (9/10)	5,65 (4/6)	—
Ductus deferens	Rat	pSm	5,33 (6/8)	— (0/6)	5,70 (4/6)
		pB75	4,89 (5/8)	5,59 (4/6)	5,26 (4/6)
		pB50	4,15 (3/8)	5,45 (4/6)	5,00 (2/6)

Sensitivity of the organs toward noradrenaline taken from the oxotremorine treated animals were increased. There was more pronounced biphasic effect of noradrenaline on oesophagus of animals treated with oxotremorine. Harmine pretreatment decreased sensitivity toward noradrenaline in all preparations with cholinergic nervus supply. Ductus deferens of the rat was more sensitive toward noradrenaline after harmine.

3. Infusion of atropine

Atropine induced increase followed by decrease of the effect of nerve stimulation on striated muscle of oesophagus. Responses of urinary bladder and ductus deferens of controle animals on atropine was decrease of contraction. Organs taken from oxotremorine treated animals did not change sensitivity towards atropine significantly. The same could be recorded for the organs taken from the animals treated with harmine.

Tab. 3.

THE INFLUENCE OF SLOW INFUSION OF ATROPINE
(5×10^{-7} M/ml/min.) ON THE RESPONSE OF ISOLATED
ORGANS INDUCED BY ELECTRICAL NERVE STIMULATION

Organ	Animal		Seven days injections i. p.		
			Saline	Oxotremorine	Harmine
Oesophagus	Rat	pSm	5,09 (5/6)	5,07 (4/6)	5,41 (4/6)
		pB75	4,72 (4/6)	4,88 (2/6)	— (0/6)
		pB50	4,62 (2/6)	— (0/6)	— (0/6)
	Mouse	pSm	5,71 (4/6)	5,71 (4/6)	—
		pB75	5,27 (5/6)	— (0/6)	—
		pB50	— (0/6)	— (0/6)	—
Urinary bladder	Rat	pSm	— (0/10)	— (0/6)	— (0/6)
		pB75	5,70 (10/10)	5,71 (4/6)	5,67 (4/6)
		pB50	5,24 (9/10)	5,32 (4/6)	5,30 (2/6)
	Mouse	pSm	— (0/8)	— (0/6)	—
		pB75	5,89 (8/8)	5,81 (6/6)	—
		pB50	5,66 (8/8)	5,59 (6/6)	—
Ductus deferens	Rat	pSm	5,11 (3/7)	— (0/6)	— (0/6)
		pB75	5,37 (6/7)	— (0/6)	— (0/6)
		pB50	5,27 (5/7)	5,57 (4/6)	5,85 (4/6)

DISCUSSION

The marked qualitative difference of the response to electrical stimulation of the nerve was not recorded on the isolated organs taken from control or test animals. The difference from the, control was found after infusion of acetylcholine and noradrenaline but not after atropine. Chronic treatment with oxotremorine provoked different change of sensitivity of organs taken from mice and rats. It was recorded species differens. Oxotremorine injected seven days one after another increased sensitivity toward acetylcholine and noradrenaline with the exception of the oesophagus of the mice, where was found decrease of the responses.

Chronic treatment with harmine decreased the effect of acetylcholine and noradrenaline on the response of nerve stimulation except on the ductus deferens. The very important exceptions are striated muscle of oesophagus and smooth muscle of vas deferens, former contracted upon the cholinergic vagus nerve stimulation (9) and later responded upon the adrenergic nerve stimulation (7).

Two tremogenic agents injected *in vivo* induced different change toward infusion of drug *in vitro*. It was recorded species differens not only in control animals (16) but in the reactions after oxotremorine and harmine toward acetylcholine and noradrenaline. The results can not be put together and analysed simply. The changes are not in the same direction and slike. The increased sensitivity could be explained by the damage and atrophy of muscle (10) after chronic treatment with oxotremorine. Decreased sensitivity can be explained by hyperactivity, for it is known, the sensitivity can be greatly reduced within a few hours only by activity of the muscle fibre (15), as was induced by tremogenic substances. It is very probable that the mechanism and the site of activity of tremogenic substances in periphery is not single ones as well for different agents as for single tremogen.

SUMMARY

The isolated organs with nerves were taken from control and treated rats and mice. Oxotremorine and harmine were injected previously seven days one after another. The nerve vere stimulated every minut with the trains of pulses and contractions of oesophagus, urinary bladder and ductus deferens were recorded. In the bath where isolate organs were set up the slow infusion of acetylcholine, noradrenaline and atropine were started, nad the change of the response were measured and expressed as a negative logarithm of molar concentrations.

The species differences were found. Oxotremorine increased sensitivity towards acetylcholine and noradrenaline with the exception of the oesophagus of mice. Harmine decreased the effect of acetylcholine and noradrenaline on the responses of nerve stimulation except ductus deferens. The two very important exceptions are striated muscle and

smooth muscle, former contracted upon the stimulation of cholinergic nerve and later upon stimulation of adrenergic nerve. The changes are not in the same directions and alike and the results can not be analysed simply. It is concluded that the mechanism and site of activity are a few in periphery.

M. RADIVOJEVIĆ I S. HUKOVIĆ

**PROMJENA OSJETLJIVOSTI PREMA ACETILHOLINU,
NORADRENALINU I ATROPINU IN VITRO POD UTICAJEM
INJICIRANJA OKSOTREMORINA I HARMINA IN VIVO**

KRATAK SADRŽAJ

Promjena osjetljivosti acetilholinskih receptora razne vrste muskulature pod uticajem supstanci i oštećenja bila je poznata odranije. Cilj ovog rada je bio da se ustanovi da li se mijenja senzitivitet prema acetilholinu, noradrenalinu i atropinu pod uticajem sedmodnevnog davanja oksotremorina i harmina. Ispitivanja su vršena na izoliranim organima sa pripadajućim nervima, uzetim od štakora i miša koji su prethodno sedam dana uzastopice injicirani oksotremorinom (0,25 mg/kg) i harminom (20 mg/kg). Nervi su stimulirani svake minute i registrovane su kontrakcije glatkih, odnosno poprečnoprugastih mišića. Upoređeni rezultati su dobiveni na kontrolnim i tretiranim preparatima i izraženi u vrijednostima p^{Sm} , p^{B75} i p^{B50} . Nije nađena velika razlika, mada postoji signifikantna razlika. Rezultati su kompleksni i ne mogu se staviti skupa i jednostavno se analizirati, jer postoje značajni izuzeci. Razlika je nađena u osjetljivosti prema acetilholinu i noradrenalinu, a ne prema atropinu. Oksotremorin povećava osjetljivost prema acetilholinu i noradrenalinu, — izuzetak je ezofagus štakora. Nađena je također razlika između vrsta, tj. drugačije se mijenja osjetljivost kod štakora nego kod miša. Harmin smanjuje osjetljivost prema acetilholinu i noradrenalinu, osim na duktus deferensu štakora. Smanjenje osjetljivosti se tumači kao posljedica hiperaktiviteta, a povećanje senzibiliteta — kao rezultat oštećenja i atrofije. Vjerovatno je da nisu jedinstveni mehanizam i mjesto djelovanja tremogenih supstanci na periferiji.

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E. S. VIZI AND J. KNOLL

THE MECHANISM OF ACETYLCHOLINE RELEASE BY NICOTINE

The distinction between »nicotinic« and »muscarinic« effects of acetylcholine, as proposed by Dale (16) more than fifty years ago, has proved its validity both for peripheral and central nervous system. Nicotine, like ACh, acts on postsynaptic cholinceptive sites and produces excitatory postsynaptic potentials (EPSP). This action can be blocked by excess of nicotine and by antinicotinic agents, such as hexamethonium and tubocurarine. The nicotine block at ganglia is associated with an initial depolarization superseded by a phase without depolarization (36).

So far, it has been accepted that nicotine acts through a cholinergic mechanism. A crucial question emerging in this problem is whether nicotine acts by mimicking acetylcholine or by releasing it or by both.

Tremor is a typical action of nicotine in both man and different species of animals. The first time in 1885 van Praag observed, that nicotine possesses this action.

Nicotine gives rise to a very shortlasting tremor, and when a second dose is administered, no tremorgenic activity can be observed (25). Also a fast tachyphylaxis was observed to the effect of nicotine in intestine.

The finding that in the longitudinal muscle strip of guinea-pig ileum all the ACh is contained in the nerve network (37), provides an opportunity for studying the release of ACh of nervous origin. Since the Auerbach plexus of ileum also contains a huge number of ganglia we studied the effect of nicotine on ACh release. Subcortex-cortex and cortex slice preparations were also used to study the site of action of nicotine and that of oxotremorine.

Methods. Longitudinal muscle strip of guinea-pig ileum was prepared as it was described by Paton and Vizi (39). Field stimulation was used. The strips were incubated at 35°C in Krebs solution containing eserine sulphate in a concentration of 2×10^{-6} g/ml. 5 strips weighing each 50—60 mg were mounted together in an organ bath of 3,5 ml capacity.

Subcortex-cortex preparation. Rats weighing 150—200 g were used. The animal was stunned by a blow on the neck and immediately decapitated. The brain was removed as rapidly as possible (6—10 sec) and placed in a moist atmosphere. Wedge-shaped slice from the brain

were cut with a blade. The slices weighing 200 — 250 mg contained some part of the thalamus, reticular system and cortex (Fig. 1). Two slices were incubated at 35°C in Krebs solution of 2 ml gassed with a mixture of 95% O₂ and 5% CO₂. Eserine sulphate (2×10^{-6} g/ml) was present throughout the experiments. However, when the action of oxotremorine was studied the slices in eserinated (10^{-5} g/ml) Ringer-Locke solution were incubated and bubbled with oxygen.

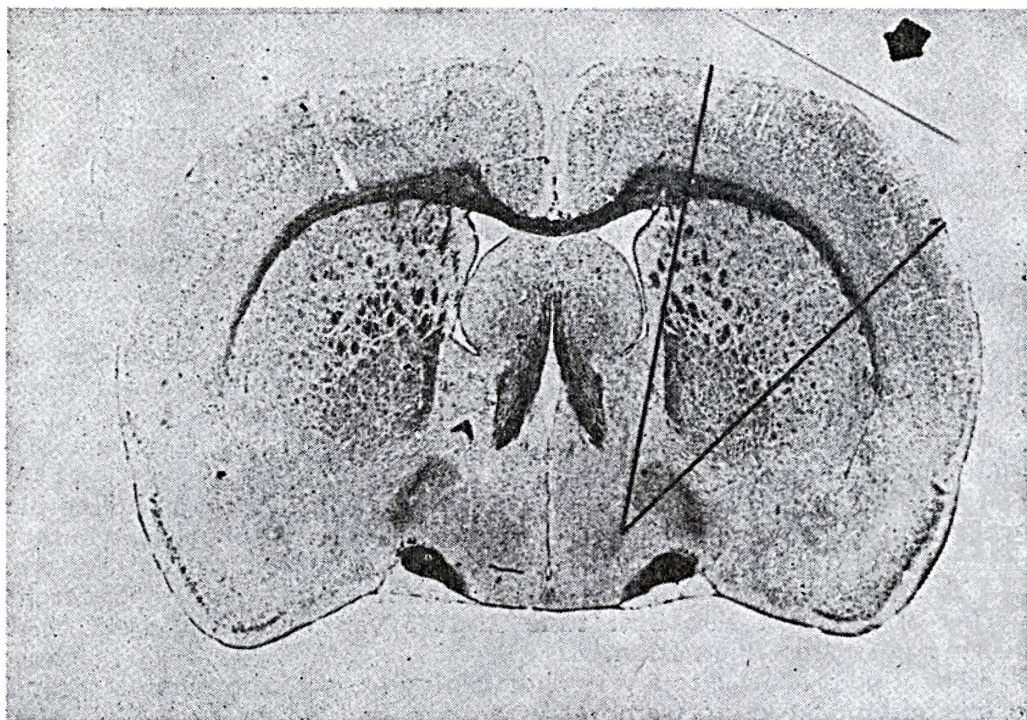


Fig. 1

Subcortex-cortex preparation. Scheme shows how the slices were prepared.

Cortical brain slice. Slices (1.5 — 2 mm thickness) were cut from the cortical surface of the rat brain.

Assay of acetylcholine. To assay ACh a length of ileum was removed from the guinea-pig. The whole ileum was used, a thread being tied at the oral end for attachment to the isometric transducer system via phosphor-bronze spring (see Fig. 2a) and another to the aboral end connecting to an open-ended polythene tube which projected through the bottom of the organ bath to allow the luminal contents to be excrudded from the ileum without contaminating the bath. The contractions were recorded by means of an isometric recording system. A transistor differential amplifier was used to overcome the drift problem and to get a high gain for driving pen recorder (Servogor). The Fig. 2a shows the block scheme of the set up for recording contractions of the guinea-pig ileum. Since prolonged isometric contraction tended to reveal spon-

taneous movements of the ileum a soft spring was used eliminate isometrical condition. In this way the sensitivity of ileum is higher and the spontaneous movement is negligible.

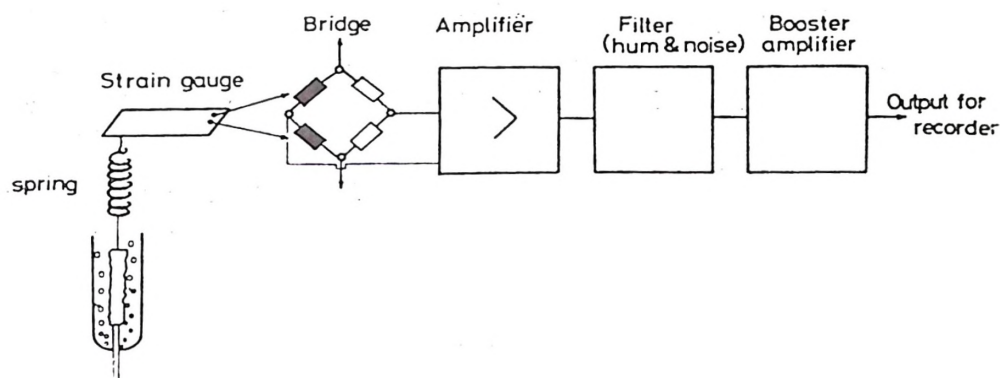
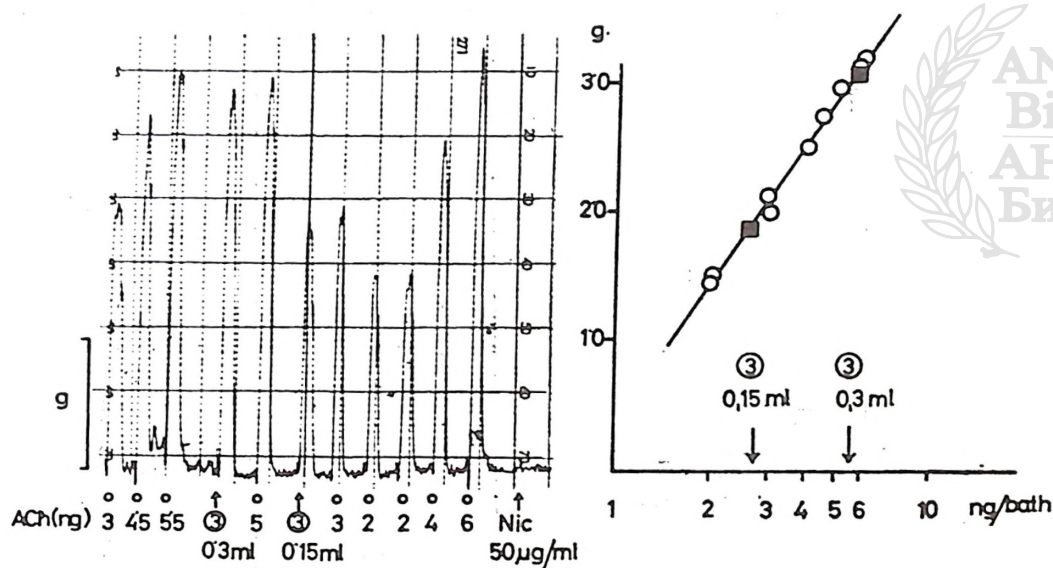


Fig. 2

a) Block-scheme of the set-up for recording the contractions of guinea-pig ileum.



In the presence of oxotremorine the ACh release has been measured in leech muscle. The leech muscle was suspended in Ringer-locke solution diluted by distilled water in a ratio of 5:7. The bath contained 10^{-5} g/ml physostigmine sulphate and 10^{-5} g/ml Morphine HCl. The sensitivity of the preparation to ACh was 2—8 ng/ml. The contractions were recorded isometrically.

Drugs used were: Acetylcholine iodide (BDH), Nicotine hydrogen tartrate (BDH), Hexamethonium bromide, /—/noradrenaline bitartrate (Fluka), Yohimbine HCl (Smith Kline and French), adrenaline HCl (Fluka), Morphinum sulphate (Alkaloida, Tiszavasvár), Tetrodotoxin (Sankyo), Mecamylamine (Erypharm Italiana), Oxotremorine esquifumarat (Loba-Chemie). The doses quoted being given in terms of their salts.

RESULTS

The effect of nicotine. It is generally accepted, that nicotine and nicotine-like drugs elicit contractions of the innervated guinea-pig ileum and of the intestine of other species by acting indirectly through the postganglionic parasympathetic nerve pathways (19, 37, 17, 1, 20). In addition, Paton and Zar (37) presented evidence for the indirect action

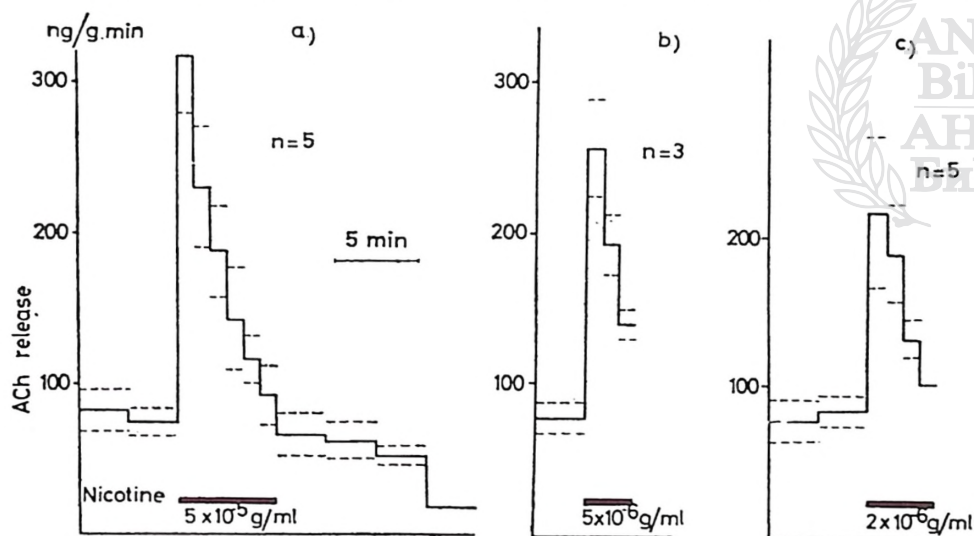


Fig. 3

ACh-releasing effect of nicotine. Long. muscle strip of guinea-pig ileum (3—5 strips, total weight 200—350 mg). The accumulated sample during the collection period (1 min when the strip was exposed to nicotine) was withdrawn entirely from the bath and the bath refilled immediately with fresh eserized Krebs-solution. Total ACh release is expressed in ng/g. min, and the standard error of the mean is indicated by dotted lines. In the figures a, b and c n indicates the number of experiments.

Note the fast tachyphylaxis to nicotine AChI and stricotine hydrogen tartrate were used.

of nicotine, showing that nicotine and DMPP failed to produce contractions in denervated smooth muscle. Their actions are also inhibited by local anaesthetic in concentrations which selectively block axonal conduction, by tetrodotoxin (20) or by ganglion blocking agents.

Fig. 3. shows the effect of nicotine added to the organ bath on the release of ACh from the nerve elements of longitudinal muscle strip of guinea-pig ileum. A concentration of 5×10^{-5} g/ml increased the resting release of ACh from 74.2 ± 10.2 to 318.5 ± 38.8 ng/g.min ($n=5$; $p<0.01$). The increases by concentrations of 5×10^{-6} and 2×10^{-6} g/ml, respectively, were also significant ($p<0.01$ in both cases). There was a very fast tachyphylaxis to the ACh-releasing action of nicotine (Fig. 3 and 4). The half time of decrease was 3,5 min in all of the three cases. If tachyphylaxis produced by nicotine represents the lack of ACh relea-

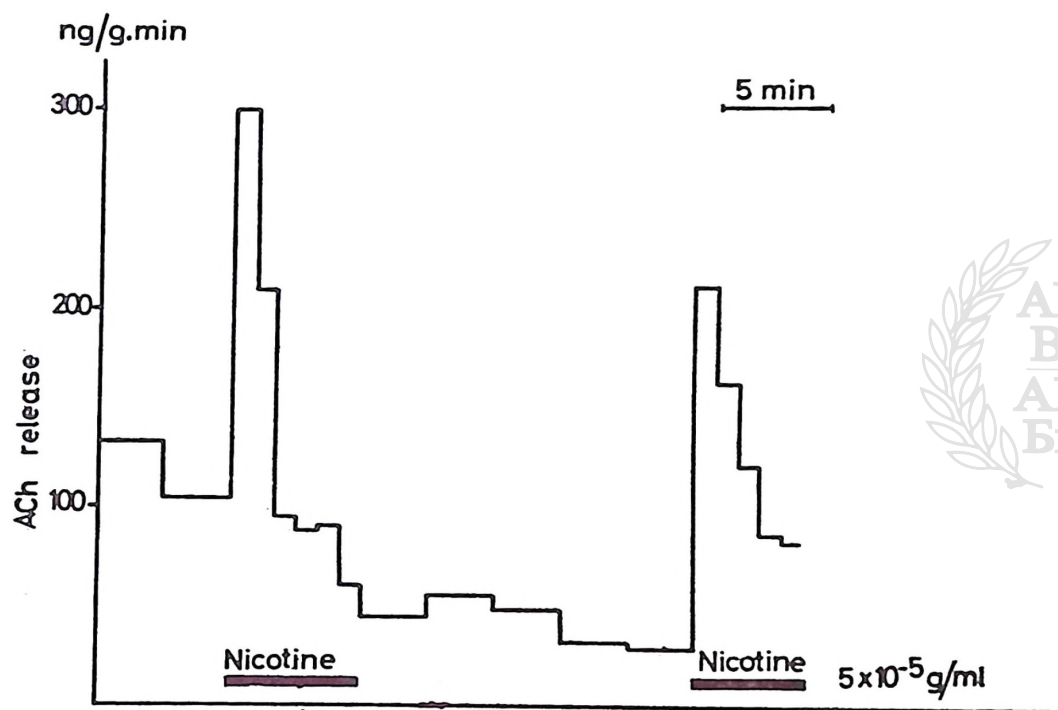


Fig. 4

ACh-releasing action of nicotine. Long. m. strip of guinea-pig ileum (5 strips in the donor bath). Average of two experiments with identical schedule. Otherwise see fig. 3.

sable from the nerve terminals, then our experiments do not exclude the possibility that nicotine releases ACh from the nerve terminals by a presynaptic action, as was suggested by Chiou et al. (7, 8). However, it seems more likely that this reduction in ACh release represents a ganglion blocking action of nicotine on ganglion cells, competitively inhibiting its own effect, since direct depolarization by high (K^+) of nerve terminals still releases ACh (Fig. 5). When the effect of nicotine

was reduced i. e. still tachyphylaxis developed, postganglionic stimulation was effective (Fig. 6). These facts indicate, that during nicotine tachyphylaxis ACh from the nerve terminals is still releasable, and the stores are not exhausted. The tachyphylaxis already developed to nicotine disappeared within 5 — 10 min after washing out the tissue (Fig. 4). It is worth mentioning that the ACh output per minute de-

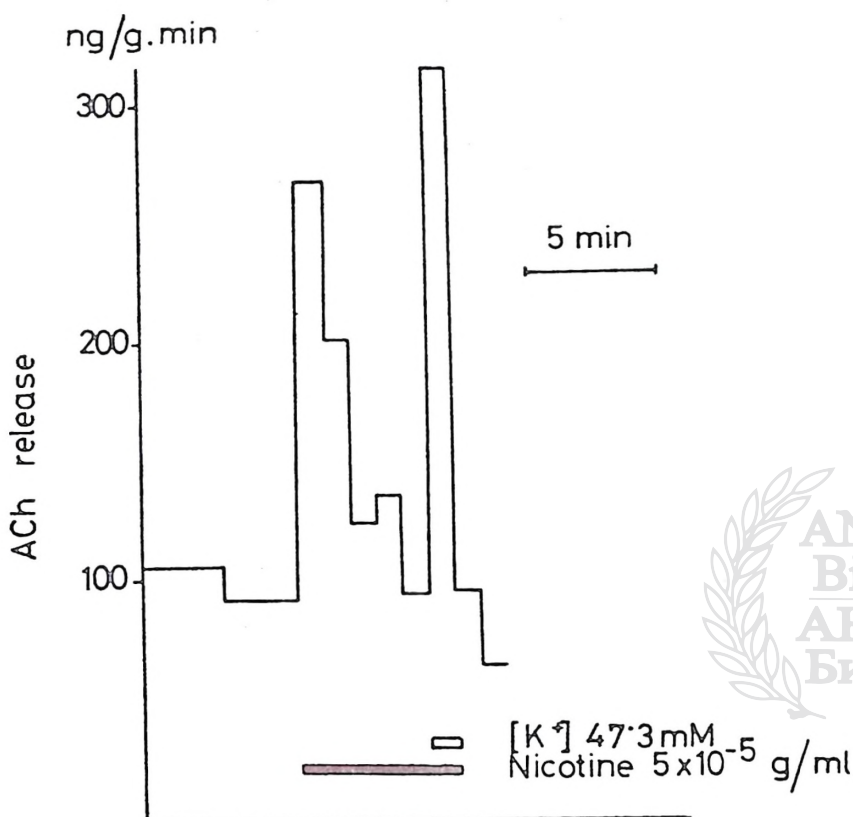


Fig. 5
The effectiveness of high K⁺/_o (47.3 mM) in releasing ACh during nicotine tachyphylaxis. Long. m. strip of guinea-pig ileum. When KCl was increased the NaCl was decreased to maintain isoosmolarity. Average of two experiments with identical schedule.

depends on the exposure time, since nicotine is able to build up tachyphylaxis. The ACh releases by longitudinal muscle strips of guinea-pigs were compared when 5 collection periods of 1 min and when 1 collection period of 5 min were used (Fig. 3 and 7a). The average of ACh output per minute did not differ; they were 198,9 (Fig. 3a) and 204,5 ng/g min (Fig. 7a) respectively.

Tetrodotoxin (5×10^{-7} g/ml), adrenaline (5×10^{-7} g/ml), noradrenaline (10^{-6} g/ml) and hexamethonium (3×10^{-4} g/ml) completely prevented the action of nicotine administered in a concentration of 5×10^{-5} g/ml (Fig. 7, 8). Morphine 10^{-6} g/ml also reduced the effect of nicotine on ACh release, from 318,8 to 146,2 ng/g min (Fig. 8).

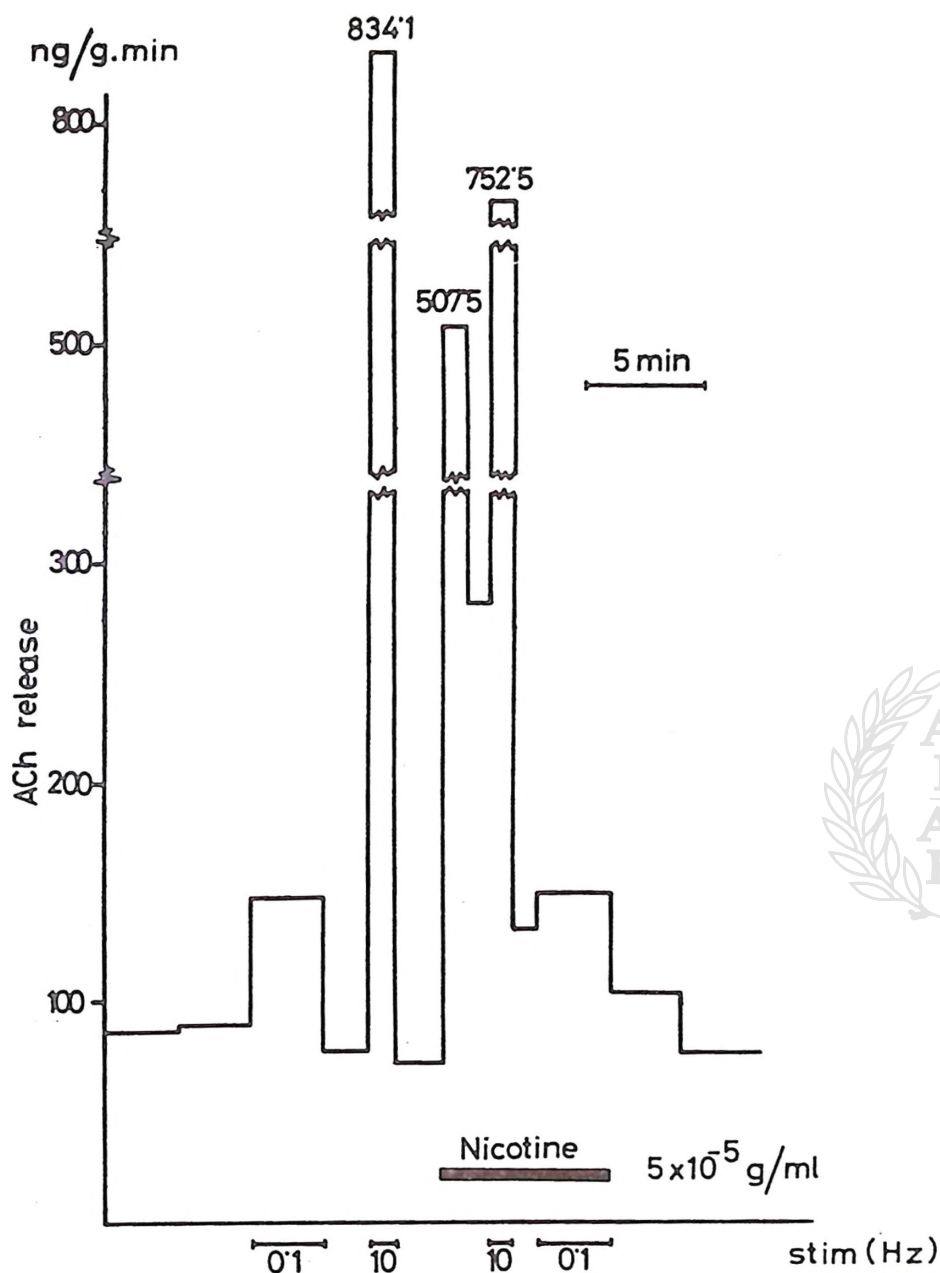


Fig. 6

ACh release by nicotine and electrical stimulation from the Auerbach plexus of long. m. strip of guinea-pig ileum. Supramaximal field stimulation was delivered using DISA Biostim at frequencies of 0.1 and 10 Hz. The length of train is indicated. Impulse duration is 1 msec.

It is interesting to note, that C_6 which reduces the ACh resting release from longitudinal muscle strip preparation during long applica-

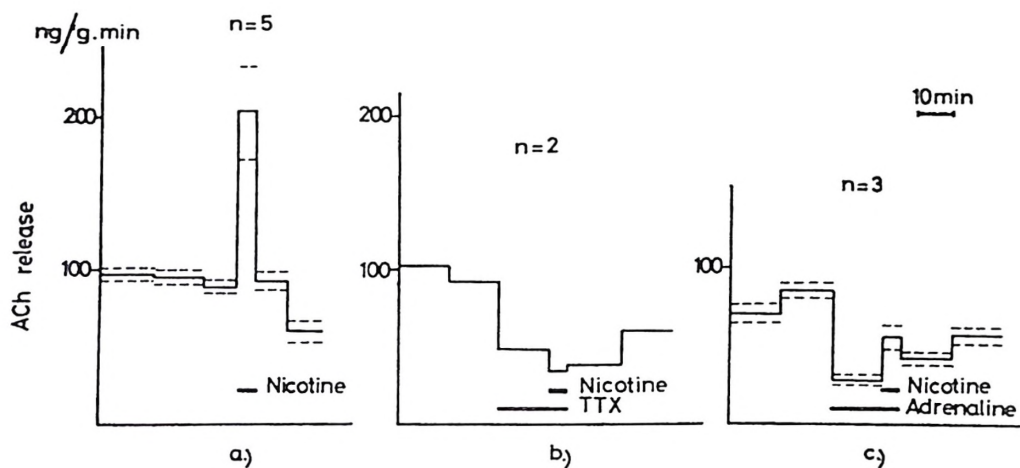


Fig. 7

The inhibition by tetrodotoxin (5×10^{-7} g/ml) or by adrenaline (5×10^{-7} g/ml) of ACh release enhanced by nicotine (5×10^{-5} g/ml). Long. m. strip of guinea-pig ileum.

tion (Paton, Vizi and Zar, unpublished) failed to reduce the ACh release, when the exposure time was as short as 3 min. However, in the next 3 min collection period there was an inhibition of 50%. When C_6 and nicotine were added together to the bath for one min, there was no reduction in ACh release. However, pretreatment by C_6 completely abolished the action of nicotine (Fig. 8).

Nicotine (5×10^{-6} g/ml) added to the bath containing cortex preparation of the rat, failed to increase the ACh release. However, when it was applied to the subcortex-cortex preparation, the ACh release was increased from 3.3 ± 0.46 to 6.7 ± 0.6 ng/g. min (Fig. 9). Since there are no true nicotinic receptors in the cortex (32) and in our experiments nicotine did not enhance the ACh release, the site of action of nicotine appears to be in the subcortex. The cell bodies of the great majority of corticocholinergic fibres are situated subcortically (23). Thus, subcortical stimulation of nicotinic receptors by nicotine resulted in the increase of ACh release. It is difficult to say whether the ascending cholinergic nonspecific reticulocortical system (28, 9, 10) and/or thalamocortical pathways (9, 10) are totally involved in our preparation, but at least some parts of them must be involved. In addition, Armistage et al. (2) have observed that nicotine applied topically to the parietal cortex had no effect on the EEG in concentrations greater than those likely to reach the same site following repeated i. v. administration. However, on its iv. administration there was a desynchronization. This experiment also indicates that there are no nicotine sensitive receptors in the cortex. In further series of experiments we have found that the increased ACh release by nicotine from subcortex-cortex preparation was inhibited by TTX (6×10^{-7} g/ml), by mecamylamine (10^{-5} g/ml) and by adrenaline (10^{-6} g/ml). The inhibition by noradre-

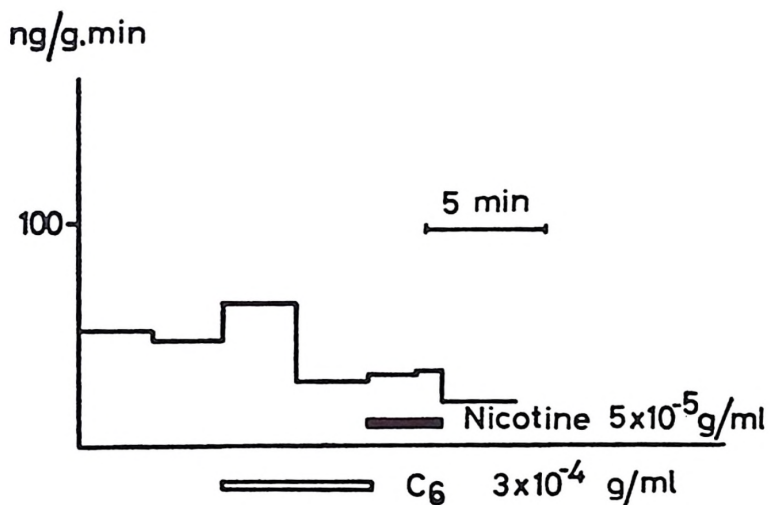
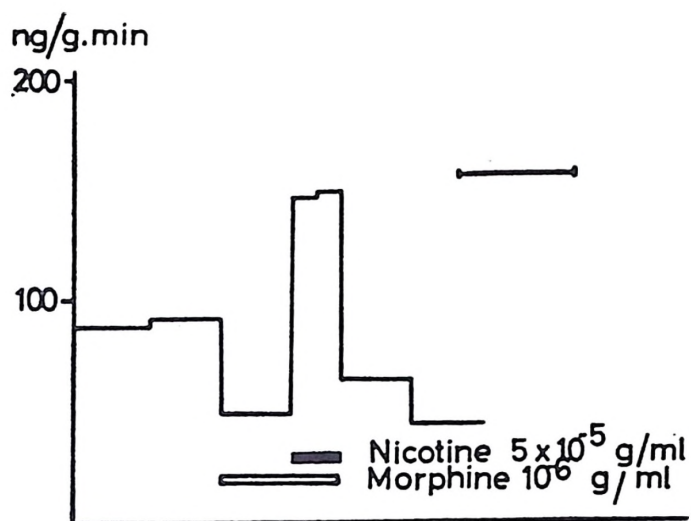


Fig. 8

The inhibition by morphine (10^{-6} g/ml) or by hexamethonium (C₆; 3×10^{-5} g/ml) of ACh release enhanced by nicotine (5×10^{-5} g/ml). Long. m. strip of guinea-pig ileum. Morphine sulphate, hexamethonium bromide and nicotine hydrogen tartrate were used.

naline of ACh release induced by nicotine was prevented by yohimbine (Table I). The ACh release augmented by nicotine (from 65.5 ± 7.1 to 245.4 ± 26.0 ng/g. min) was inhibited when noradrenaline in a concen-

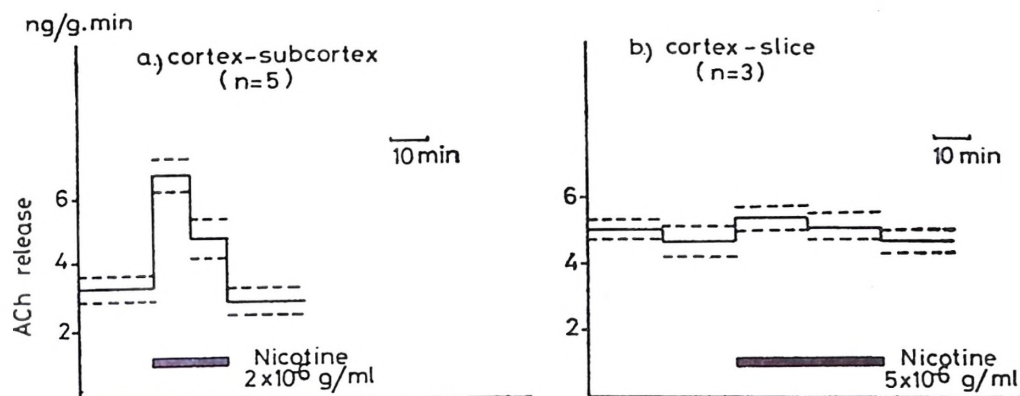


Fig. 9

The effect of nicotine on ACh release from subcortex-cortex and from cortex slice preparation of the at. Eserinized Krebs solution, 2 ml.

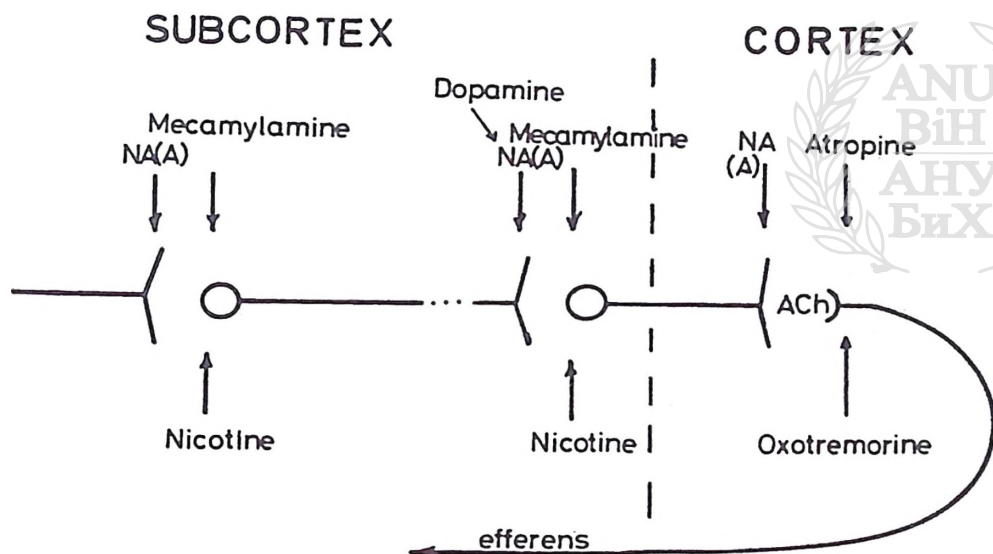


Fig. 10

The site of action of nicotine in the central nervous system.

tration of 2×10^{-6} g/ml was added to the bath; 84.2 ng/g. min was the release of ACh. However, when the strips were exposed to yohimbine (3×10^{-6} g/ml) for 20 min, there was a complete prevention of inhibition by noradrenaline (Table I). Our experiments indicate the role of α -receptor in the inhibitory action of noradrenaline on ACh release.

Table I

THE ROLE OF α -RECEPTOR IN THE INHIBITION BY NORADRENALINE OF NICOTINE EFFECT ON ACETYLCHOLINE RELEASE. LONGITUDINAL MUSCLE STRIP OF GUINEA-PIG ILEUM

conditions	ACh release ng/g. min	signif.	
1. Control (5)	65.5 $\pm$ 7.1		
2. Nicotine (4) 5 $\times 10^{-6}$ g/ml	245.4 $\pm$ 26.0	2:1	p < 0.01
3. (—)-noradrenaline (3) 2 $\times 10^{-6}$ g/ml	16.1 $\pm$ 3.4	3:1	p < 0.01
4. (—)-noradrenaline 2 $\times 10^{-6}$ g/ml + nicotine 5 $\times 10^{-6}$ g/ml (4)	60.5 $\pm$ 21.1	4:6	p < 0.01
5. Yohimbine 30 $\times 10^{-6}$ g/ml + (—)-noradrenaline 2 $\times 10^{-6}$ g/ml (5)	84.2 $\pm$ 14.5	5:3 5:1	p < 0.01 p > 0.05
6. (—)-noradrenaline (3) 2 $\times 10^{-6}$ g/ml + Yohimbine 30 $\times 10^{-6}$ g/ml + nicotine 5 $\times 10^{-6}$ g/ml	191.5 $\pm$ 14.8	6:4 6:1	p < 0.01 p < 0.01

The exposure time to nicotine is 1 min, at Yohimbine administration 20 min. Collection time during control = 3 min. Yohimbine HCl, (—)-noradrenaline bitartrate and nicotine hydrogen tartrate were used.

The effect of oxotremorine

It has been suggested that the tremorigenic action of tremorine is due to its capability of releasing ACh (24). The indirect effect of oxotremorine was also suggested by Crossland and Slater (15) and György et al. (21). Cox and Potkonjak (12), however, found that after oxotremorine, the time course of the tremor and the time course of the increase in whole brain ACh level differed markedly. Furthermore they also observed that the tremor induced by oxotremorine was suspended by atropine in a dose which did not prevent the oxotremorine-induced increase in whole ACh brain level. Dyflos, a strong cholinesterase inhibitor, failed to potentiate the tremorigenic action of oxotremorine (13). Keranen et al. (29) observed only summation of the tremorigenic action of tremorine and physostigmine, and some antagonism between neostigmine and tremorine. Also physostigmine failed to increase the effect of tremorine on temperature.

If the indirect action of oxotremorine were the case, one would have to assume, that the oxotremorine increases the ACh release. However, direct measurement of ACh release from Auerbach plexus and from cortex slice of the rat failed to support this hypothesis (26). Oxotremorine (10^{-4} M) did not increase the ACh release from the Auerbach plexus of guinea-pig intestine, even decreased from 21.8 ± 3.8 ng/g. min to 14.1 ± 4.1 ng/g. min ($p < 0.05$). Oxotremorine also failed to increase

Table II
THE EFFECT OF OXOTREMORINE ON ACh RELEASE

	ACh release* ng/g. min	signif
Control (7) Oxotremorine (7) 10 ⁻⁴ M	21.8 ± 3.8 14.1 ± 4.1	} p < 0,05 long. m. strip of. g. p. ileum
Control (3) Oxotremorine (3) 10 ⁻⁵ M	4.44 ± 1.3 4.86 ± 0.5	} n. s. } cortex slice of the rat
Control (3) Oxotremorine (3) 10 ⁻⁴ M	4.01 ± 0.25 3.53 ± 0.15	

* ACh release during the collection period of 60 min. In brackets are indicated the number of experiments.

the ACh release from cortex-slice of the rat (Table II). Our findings are in agreement with those of Cox and Hecker (54) who also presented evidence that oxotremorine acts directly on the muscarinic receptors and did not release ACh from the guinea-pig ileum. The fast tolerance to some of the effects of oxotremorine was also attributed to catecholamines (22).

DISCUSSION

Until recently the evidence for the acetylcholine releasing action of nicotine was indirect, and was based upon the fact that drugs like atropine, hexamethonium, tetrodotoxin etc. can inhibit its pharmacological actions.

In these experiments a highly significant increase by nicotine of ACh release was observed. The ACh release was measured directly. Noradrenaline, adrenaline, tetrodotoxin, hexamethonium prevented and morphine reduced the effect of nicotine on ACh release. Atropine has already been shown to have an inhibitory effect against physostigmine-induced tremors, while generally inhibiting nicotine-induced tremors only in much greater doses.

Taking into account our results presented in this paper it is highly probable, that the »muscarinic« tremor (e. g. produced by physostigmine or oxotremorine) and the »nicotinic« tremor is similar in mechanism. Both are produced by increased saturation of muscarinic receptors by ACh, but in the case of nicotine it is caused by increased ACh release *in loco*. However, there is a great difference in antagonism. The nicotine depolarizes the cell bodies which in turn releases a high amount of ACh *in loco* at receptor areas, resulting in tremor. This can more easily be inhibited by antinicotinic agents than by atropine since the concen-

tration of agonist released is very high in a minute time unit. The depolarization by nicotine can be more easily inhibited, than to prevent the effect of already released ACh at receptor sites. The problem is similar to that of antagonism existing between endogenous (released), exogenous noradrenaline and α -blocking agents. The effect of exogenous noradrenaline can be more easily blocked than that of NA released to at nerve terminals.

It appears likely that the fast tolerance to nicotine-induced tremor (25, 6) is strongly related to the tachyphylaxis observed with ACh release.

Holmstedt and Lundgren (24, 25) have demonstrated that the tremorgenic agents with muscarin-like activity increase the ACh content of the total brain, while nicotine fails to change the content (25). Although, oxotremorine is considered an indirect acting tremorgenic agent (24), in our experiments it failed to release ACh measured directly.

Noradrenaline (adrenaline) was capable of inhibiting the effect of nicotine to increase ACh release in the Auerbach plexus of the longitudinal muscle strip and in cortex-subcortex preparation. The action of NA (A) to reduce ACh release induced by stimulation is situated presynaptically, as was shown by Paton and Vizi, (39); Vizi, (43, 44); Knoll and Vizi, (30, 31).

In addition, the effectiveness of dopamine and adrenaline (11, 24) in suppressing tremor following intracaudate injection of ChE-inhibition or carbachol can be attributed to the inhibitory effect of catecholamines on ACh release (39, 43, 44, 30, 31).

The inhibitory action of noradrenaline on ACh release induced by nicotine was prevented by Yohimbine, indicating that the α -receptor plays some role.

The role of α -receptor was also established by Paton and Vizi (39) showing that the inhibition by noradrenaline of ACh output induced by nerve stimulation was prevented by α -blocking agents. Our results with nicotine can probably explain the observations of McDougal and West (35), who found that the contraction of longitudinal smooth muscle produced by nicotine can be reduced by doses of noradrenaline and adrenaline which have little or no effect on the contraction produced by histamine and acetylcholine. Furthermore, they found that sympatholytic agents prevented the effect of adrenaline in antagonizing the nicotine contraction. I. e. nicotine exerts its effect through ACh release and noradrenaline can inhibit the release.

The failure of nicotine to increase the ACh release in the presence of C_6 also favours the postsynaptic action of nicotine on nicotinic receptors (Fig. 8). Brown (4, 5) presented evidences that ACh and even nicotinic stimulants (carbachol, TMA, nicotine) are not capable of releasing ACh from the presynaptic nerve terminals of the superior cervical ganglion of the cat. The slight inhibitory action of C_6 on Nicotine uptake by the ganglion (3) cannot be activated inhibitory effect on the nicotine induced depolarization. Chiou and his coworkers (8) have shown, that nicotinic agents such as nicotine, TMA, carbachol release ACh efficiently from the nerve terminals and from rat cerebral cortex synaptic vesicles. These results suggest that the nicotinic agents are taken to an intracel-

luar site of the nerve tissues and release ACh from the synaptic vesicles, i. e. a presynaptic site of action of nicotine and nicotinic agents is concluded. However in order to observe any significant release of ACh by nicotine from isolated vesicles, Chiou had to use nicotine in a concentration of at least 100 ug/ml.

However, in our experiments nicotine was effective in releasing ACh as low as $2-5 \times 10^{-6}$ g/ml concentrations. Feldberg and Vartiainen (18) perfused the superior cervical ganglion of the cat in situ with a solution containing nicotine. The perfusate was found to be free from ACh on bioassay. TTX prevented the effect of nicotine in releasing ACh. Gershon (20) also observed that TTX blocks the contraction caused by nicotine in intestine. These facts also indicate that nicotine itself do not release ACh from the presynaptic (prejunctional) nerve terminals, i. e. the site of action is not on the nerve terminals.

Nicotine was able to release ACh from the subcortex-cortex preparation and the Auerbach plexus which is rich in ganglia. One can therefore reasonably conclude that nicotine excites the cell bodies of subcortical afferent neurons which in turn leads to an ACh release (see Fig. 10) from the cortex nerve terminals. The increased release of ACh can evoke (via muscarinic receptors and via efferent neurons) symptoms which are characteristic of enhanced cholinergic function (tremor, convulsions etc.) It can be concluded, therefore, that the tremor, convulsions, behavioural actions and the peripheral actions induced by nicotine is highly probably due to the released ACh acting on muscarinic receptors. As regards its complex action, its releasing effect on other transmitters, can not be excluded.

The fact that noradrenaline and adrenaline could inhibit the ACh release augmented by nicotine gives a further evidence that the adrenergic system plays a very important role in cholinergic system by inhibiting the ACh release presynaptically as was suggested by Paton and Vizi (39) and Knoll and Vizi (30, 31). Since the imbalance between adrenergic and cholinergic system by reserpine or by phenoxybenzamine pretreatment led to an increased ACh release (39, 44) the Parkinson-syndrome induced by drugs which depress adrenergic function either by reducing the output of NA, such as reserpine, tetrabenazine or by antagonizing its action, as for instance phenoxybenzamine, haloperidol, chlorpromazine, can be attributed to the augmented ACh release. The reduction of adrenergic outflow has to be central in origin since Jellic and Stern (27) have shown, that the peripheral sympathetic nervous system has no effect on the tremor.

Nicotine proved to be a very useful tool for studying the action of different drugs on the ACh release from nerve terminals. Nicotine mimicks the physiological transmission process depolarizing the nerve cells which in turn leads to an increase of ACh release.

Acknowledgment. We are particularly indebted to Miss Maria Simon for highly skilled technical assistance and for an unusual devotion to this research.

O MEHANIZMU OSLOBAĐANJA ACETILHOLINA POMOĆU NIKOTINA

KRATAK SADRŽAJ

Proučavano je djeystvo nikotina, jednog od najšire upotrebljavanog tremorgeničkog agensa, na oslobađanje acetilholina (ACh). Oslobođeni acetilholin je prikupljen u Krebsovoj otopini na 37°C i sadržavao je ezerin sulfat (2×10^{-6} g/ml), a zatim je isproban na ileumu zamorčeta uz prisustvo hexametoniuma (C_6 ; 3×10^{-4} g/ml). Ostatak oslobođenog ACh iz Auerbach plexusa longitudinalne mišićne niti ileuma zamorčeta bio je 74.2 ng/g. min. Ako je bio dodan nikotin (5×10^{-5} g/ml), oslobađanje se povećalo na 318.5 ng/min. Ipak, tetrodotoxin (5×10^{-7} g/ml), adrenalin (5×10^{-7} g/ml), noradrenalin (10^{-6} g/ml), morfin (10^{-6} g/ml) i C_6 (10^{-4} g/ml) zaustavili su djeystvo nikotina na povećanje oslobađanja ACh.

Nikotin (5×10^{-5} g/ml) nije uspio da poveća oslobađanje ACh iz korteksa štakora. Ipak, nikotin je udvostručio oslobađanje (5.6 ng/g. min) iz korteks-subkorteks preparata in vitro. Tetrodotoxin (3×10^{-7} g/ml) i adrenalin (10^{-6} g/ml) inhibirali su djeystvo nikotina. Razvila se brza tolerancija na djeystvo nikotina da oslobađa ACh dok se davao u dozama 5×10^{-5} g/ml. Ovo djeystvo nikotina na oslobađanje ACh može se pripisati njegovom depolarizirajućem djeystvu na ganglijske ili nervne ćelije, što u turnusu dovodi do oslobađanja ACh iz nervnih završetaka. Budući da je aktivizacija muskarinskih ACh receptora neizvjesna u pogledu na stvaranje tremora, ispitaće se uloga oslobađanja ACh u tremogeničkom djeystvu nikotina. Vrijedi napomenuti da oksotremorin nije uspio povećati oslobađanje ACh.

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DISCUSSION

Stern. I think that nicotine does'nt produce tremor. However, I have never seen it.

Vizi: Well, one thing is sure, that nicotine does not produce a long-lasting tremor. But it does evoke a tremor which lasts for a short time. The short-lasting tremor by nicotine is probable correlated with the fact tachyphylaxis to nicotine observed in ACh-release. I am agreeing with Professor Stern that nicotine not the best drug in producing tremor nevertheless, it causes tremor but short in time (Holmstedt and Lundgren, Ann. N. Y. Acad. Sci. 142: 126—142, 1967).

Župančič: Doctor Vizi, you said that hexamethonium prevents nicotine to release acetylcholine, right? Next, I understood you found this result unexpected, and I wonder just why would you like to comment on this point.

Vizi: I have a feeling this was misunderstood. Not the inhibition is unexpected the time course.

Barbeau: I would like to confirm two important remarks made by Prof. Vizi: 1) Dopamine does inhibit release of ACh, especially in the striatum and this is one of the most important control mechanisms. 2) You rightly emphasize the importance of muscarinic receptor site saturation for the production of tremor. The receptor has been the forgotten part of this mechanism. Lastly a question: instead of mimicking ACh could it be that oxotremorine changes the sensitivity of the receptor site or possibly of the »Target« cell?

Vizi: I am glad that Prof. Barbeau is agreeing with our results and suggestions. Yes, in one experiment Dopamine reduced by 50% the volley output (ACh output due stimulation) of ACh from the nerve terminals of Auerbach plexus. In subcortex-cortex preparation the nicotine-induced increase of ACh release was also reduced by Dopamine. This confirms your work on the role of Dopamine in Parkinson syndrome. I do not think that oxotremorine could change the sensitivity of the receptor site. The fact that there is a competitive type of antagonism between Atropine and Oxotremorine, indicates that Oxotremorine acts on the receptor. We have studied this problem. Otherwise the $pA_2 - pA_{10}$ value which (Schield) was calculated also a little piece of evidence for believing the effect of Oxotremorine on muscarinic receptors. We do think that Oxotremorine mimicks the action of acetylcholine.

Schäfer: During the reserpin induced tremor increase of the alpha-activity was noticed, but tremorin induced tremor showed gentel gamma-activity. On

a base of different changes of sensibility of the motor system, a different forme of the tremor after inducing the drugs must be expected. Does the tremors in both case differ? Because the impulses of the alpha-motor neuron on spinal level because activity in both case can be different too. Have you researched for this?

Vizi: We did not make any attempts to distinguish reserpine and oxotremorine tremor by frequency analysis. Concerning your question on reflex excitability of alpha motoneurons, it was tested by stimulation of the dorsal roots.

Huković: Have you seen similar effects on other organs? Do you have explanation for different results with nicotine and oxotremorine considering biphasic effect on release and effect of nerve stimulation?

Vizi: If you think on ACh release I can say, yes. We have studied the ACh release in other organs, as well. We have found that e. g. was deferens of the rat contains ACh in a concentration of 2 microgr/ml (Kroll, Somogyi and Vizi, in press) and using fiels stimulation there was on increase of ACh release depending on the frequency used (Knoll et al. in press). But the action of nicotine or of oxotremorine on ACh release from the was deferens we did not study. I do not think that would be any correlation between, tachyphylaxis (to the ACh releasing action of nicotine) and »biphasic« effect of oxotremorine on the contraction of vas deferens in your experiments, since at the moment it is generally accepted that in vas deferens the NA is the transmitter. However we also believe that in vas deferens ACh might have some role in maintaining excitability of smooth muscle cells. In addition, in our Department Dr Somogyi also observed that oxotremorine (10^{-7} g/ml) increase contraction by stimulation of was deferens and that this action can be prevented by Atropine.

Bernard: It was mentioned that dopamine reduces acetylcholine release. Has L-DOPA a similar action?

Vizi: I can not tell you, because we have not tried.

Cox: Dr Vizi may I raise a few points some of which lend support to your hypothesis. Firstly oxotremorine on guinea-pig ileum is competitively antagonized by atropine when tested by the method of Arunlakshana and Schild. It is also twice as potent as ACh on this preparation. Therefore one might except it to be a potent muscarinic agonist in the CNS such as at the site you postulate. Mayi also point out the problem of measuring ACh release — »so colled« resting release differs depending on the cholinesterase inhibitor used to protect ACh. Thus release rates for eserine are much higher than release rates for mipafox (an organophosphorus inhibitor). It is quite possible that eserine may itself induce ACh release and this could affect results obtained. Finally there is the problem of facilitated or non action potential mediated relaease of ACh. The agents used tetrodotoxin and morphine will only prevent action potential released ACh. Nevertheless it seems to me that the weight of evidence favours a post-synaptic effect of oxotremorine on a central muscarinic receptor.

Vizi: I should like to make clear asfar as the »resting« release is considered. Resting release from long. m strip of guinea-pig ileum can be reduced by about 50 — 70% by TTX, Hexamethonium and by NA (A) (Paton and Vizi, Brit. J. Pharmacol. 35, 10 — 28, 1969 and Paton, Vizi and Zar, in press). This indicates that one part of the release is maintained by the activity nervous and the conduction is involved in the release. I think it is the high time to discuss about Mipafox. Paton and Zar (personal communication) found that Mipafox somehow interferes with the release mechanism. Furthermore, if I am correct, in your last paper (Cox and Hecker, Brit. J. Pharmacol. 41, 19—25, 1971) you presented evidence that the output to stimulation was very low when Mipafox was applied. These fact seem to me to strengthen the

feeling that Mipafox is not the appropriate ChE-I, when one wants to measure the ACh release by action potential. The physostigmine is not only the most frequently used ChE, but it seems to be the drug which does not influence the conduction, and the release of ACh due to nervous activity can be measured reliable. We could not found for instance difference in the action of NA/A (in reducing ACh release) either in the presence or in the absence of physostigmine. There was also a good correlation between the reduction by TTX of ACh release in the presence of physostigmine and the reduction of size of contraction induced by stimulation in long. m. strip of guinea-pig ileum.



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CHE ACTIVITY IN WHOLE BRAIN AND BASAL GANGLIA OF RATS TREATED WITH MANGANESE

In our earlier experiments we investigated the influence of manganous chloride and manganous sulfate on ChE activity in whole brain of rats treated with manganous salts (4). The reason for this investigations was the fact that in chronical poisoning of manganous salts which may cause extrapyramidal syndrome, the role of holinergeric system is not enough investigated up to date. In our work we found in rats treated by manganous chloride and manganous sulfate increase of ChE activity in whole brain, but differences were not significant with regard to the control. Now the question is whether manganous ions present in brain of animals treated with Mn^{++} — ions may influence the ChE activity.

The subject of our present study is the investigation of influence of Mn^{++} — ions on ChE activity in homogenate of whole brain in vitro.

In addition to this our study in whole brain we carried out also the control. Now the question is whether manganous ions present in basal ganglia.

MATERIALS AND METHODS

For preparation of samples we used a supernatant of whole brain's homogenate, prepared in following sheme. Concentration of manganous chloride in examined samples was 0,20 — 0,60‰ in whole brain homogenate and 0,45 ml distilled water. The experimental group presents the following mixtures:

Samples	Brain homogenate in ml	ml of 1‰ $MnCl_2$	ml of water
A	0,30	0,15	0,30
B	0,30	0,30	0,15
C	0,30	0,45	—

Acknowledgments. We are indebted to the Federal Research Fund Belgrade for the financial support granted for this investigation.

As blank for brain homogenate was used solutiones made by following sheme:

Sample	ml 1% $MnCl_2$	ml H_2O
A	0,15	0,60
B	0,30	0,45
C	0,45	0,30

Cholinesterase activity was determined by Hestrin's method (1).

For histochemical investigations we used Wistar albino rats, female, body weight 100 — 115 g treated with manganese chloride in doses 15 mg/kg during 21 days. As control same sort of Wistar albino rats were used. In experiment brain first was washed with Ringer via left ventricle and than basal ganglia was removed. Frozen brain were cut in slides and Gomori's procedure were used for determination of ChE activity (3). Incubation medium were prepared fresh and for preincubation we used three specific inhibitors — iso-OMPA in concentration 1×10^{-4} , BW 284 C 51 in concentration of 1×10^{-5} and eserine in concentration 1×10^{-5} (2). Preincubation was carried out in medium without supstrate when inhibitor was not added during 15 minutes. As supstrates AThCh and BuThCh were used.

RESULTS

The results have presented in table 1—3.

Table 1.

EFFECTS OF Mn^{++} — IONS ON ChE ACTIVITY IN BRAIN HOMOGENATE

Samples	Experimental group N-4	Control N-4
	1	2
A	72,54	7,05 = 100%
B	67,03	
C	62,57	

1. ChE activity in percentage of the mean value of the control.

2. The mean value of ChE activity = 100%

The brain homogenate exposed to Mn^{++} — ions one hour.

The decrease of ChE activity in the brain homogenate treated with Mn^{++} — ions is evident.

The distribution of enzyme activity in basal ganglia is presented in table 2.

Table 2.
ACTIVITY OF AChE IN RATS BASALE GANGLIA

Experiments	Localisation and AChE-activity		
	Nuc. caudatus	Putamen	Gl. pallidus
1. Incub. medium without the addition of substrate	—	—	—
2. Incub. medium+substrate AThCh	++	++	+
3. Incub. med. + isoOMPA 10^{-4} + substrate AThCh	++	++	+
4. Incub. med. + BW 10^{-5} + AThCh	—	—	—
5. Incub. med. + eserine 10^{-5} + AThCh	—	—	—
6. Incub. med. + BuThCh	+	+	+
7. Incub. med. + iso-OMPA 10^{-4} + BuThCh	—	—	—
8. Incub. med. + BW 10^{-5} + BuThCh	+	+	+
9. Incub. med. + eserine 10^{-5} + BuThCh	—	—	—

From these histochemical results we can make conclusion that the incubation medium with substrate gives characteristic color reaction, which was more intensive with AThCh than by BuThCh.

With specifics inhibitors of AChE, BW284C51 and eserine color reaction was not expressed. By using inhibitors of BuChE, iso-OMPA and eserine the tissue was without the color reaction, and by BW284C51 inhibitors color reaction was observed.

Color intensity was more pronounced in Putamen and nucleus caudatus than in Gl. pallidus, as had been noted in literature. The tissue color in the basal nuclei was more times intensive than in the cortex (Fig. 1).

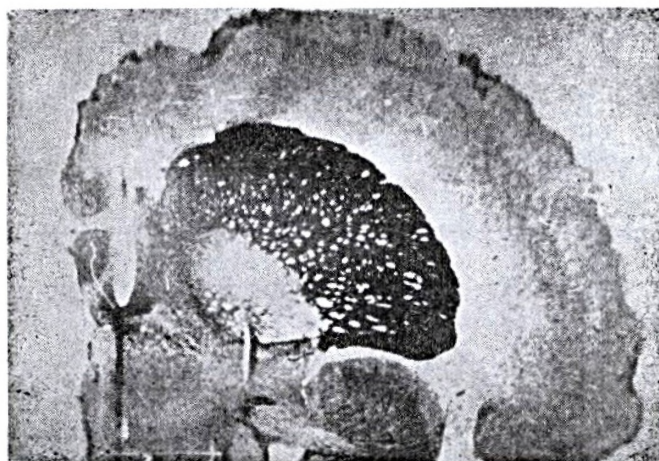
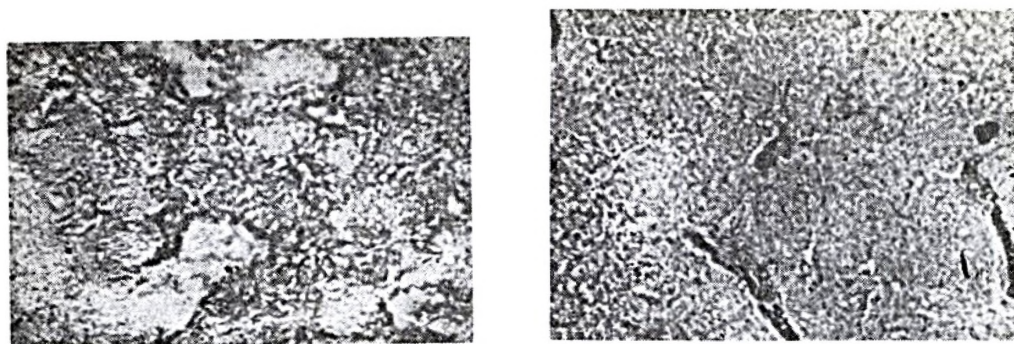


Fig. 1

The distribution of ChE in basal ganglia and cortex.

Localisation of AChE and BuChE in basal ganglia was quite different. AChE were in neuropil and BuChE were localised in the neuron cells and dendrites (Fig. 2, a and b). The intensity of tissue color of examined group comparison to control was not significantly different.



a)

b)

Fig. 2

a) AChE localisation in neuropil,

b) BuChE localisation in dendrites and neuron.

The enzyme activity was determined with Warburg respirometer. This results are in agreement with histochemical findings. The yielded activity ChE is higher, than BuChE (Table 3).

Table 3.

ACTIVITY AChE and BuChE IN BASAL GANGLIA

№ Sample	Substrates			
	AThCh	BuThCh		
	Experimental group in % of control	Control ml CO ₂	Experimental group in % of control	Control ml CO ₂
1	124,86	Average=354,66	106,33	Average=24,45
2	121,04		139,14	
3	117,47		98,15	
4	120,77		84,82	
5	116,17		97,90	
Average = 120,06		100%	Average = 105,44	100%

CONCLUSIONS

1. Mn^{++} — ions inhibit ChE activity in brain's homogenate of rats in vitro.
2. Corelation between decrease of ChE activity and concentration of Mn^{++} — ions could be established.
3. The discordance in results noted in paper as regarding ChE activity in the brain tissue points at possibility that there are probably in the brain some regions with high and other with low or without ChE activity. Therefore it would be necessary in animals treated with manganese to make detail mapping of ChE activity in brain.

AKTIVNOST HOLINESTERAZE U MOZGU I BAZALNIM GANGLIJAMA PACOVA TRETIRANIH MANGANHLORIDOM

KRATAK SADRŽAJ

Ispitivana je aktivnost ChE u homogenatu mozga i posebno u regijama bazalnih ganglija albino pacova tretiranih manganhloridom. Uporedo s ovim istraživanjem određivana je aktivnost ChE u homogenatu mozga normalnih životinja in vitro nakon dodavanja homogenatu $MnCl_2$ u više poznatih koncentracija. Utvrđena je smanjena aktivnost ChE, iako se u potpunosti nije mogla dokazati korelacija između stepena smanjenja aktivnosti ChE i dodanih koncentracija $MnCl_2$.

U histohemijskom istraživanju aktivnosti ChE u izolovanoj regiji bazalnih ganglija nisu se sa sigurnošću mogle dokazati značajne promjene. U toku su dalja istraživanja ChE u pojedinim bazalnim ganglijama.

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DISCUSSION

Funcke: Have you seen any signs of tremor or other neurologic deficit in your manganese poisoned rats? For in monkeys manganese poisoning causes tremor.

Stanković: In our experiments in albino rats treated with $MnCl_2$ (21—30 days 15 mg/kg body weight by intraperitoneal injection) we could get rigidity without tremor. As we know, tremor with manganese was in experimental animal caused only in monkey *Macacus rhesus* (Mell, 1942, van Bogaert and Dallemagne, 1943, 1945).

Vojvodić: Do you have some evidence since you use Koelle's technic, about the relationship between functional and non-functional cholinesterase in your experimental conditions?

Pleho: 1) In experimental group expressed color which showed activity ChE by histochemical Koelle's procedure in comparison to control group was not evidently more intensive and because of it we used Warburg's quantitative method for determination of ChE activity. 2) We did not control restoration ChE activity after adding eserine. Eserine, as noted in literature, inactivated ChE activity in brain homogenate in high per cent and histochemical procedure inactivation is about 95%.

ANDRÉ BARBEAU, JOHN DONALDSON AND JIM MINNICH

TREMOR, AMINES AND TRACE METALS

Professor Stern has kindly asked me to talk on the pathophysiology of tremor as it relates to amines and trace metals. It is always difficult to understand the delicate biochemistry of any illness, but the subject becomes even more hazardous when one attempts to delineate the chemical changes in the brain underlying any one symptom. Studies on Parkinson's disease during the last 10 years (1—4) have implicated disturbances in putative neurotransmitters, particularly dopamine, serotonin, and acetylcholine. The only clear relationship with symptoms is that between dopamine and akinesia, but this statement does not imply that other transmitters are not also involved as a consequence.

Thus in attempting to delineate the biochemical substrate of tremor we are facing a challenge that probably surpasses our presently available knowledge. We will therefore confine our remarks to outlining broad experimental results and possible avenues worth exploring.

A — TREMOR

The first task we should undertake is to define in physiological terms the problem we want to solve. Doing so for tremor is not easy. Clinically, as is well known, there are numerous forms of tremor, from the slow 3—8/sec. resting alternating movements of the hands and feet, classically associated with Parkinson's disease, to the rapid, fine intention or attitude tremor occasionally involving the head, which has received the name «essential familial tremor», or to the large-amplitude «flapping» tremor of Wilson's disease and hepatic failure. If one also considers the tremor seen in delirium tremens, in hyperthyroidism, in anxiety or stress, it is difficult to imagine that these various manifestations of the phenomenon rest upon a common physiological or biochemical substratum. And yet such a proposal has been made on occasion. It is not our purpose here to review this subject in detail. Suffice it to recall that, at one time or another, every level of the neuraxis has been thought to be involved in the pathophysiology of tremor, from the gamma system of the spinal cord to the cortex. The search for a so-called «tremorigenic center» has not ended, but it now focusses mainly on

the brain stem reticular formation and a loop involving the basal ganglia, thalamus and cortex. This last proposal, best studied by the group of Cordeau, Jasper and Lamarre (5—7) calls for a reverberating circuit between these brain areas which in itself would constitute the »tremorigenic center« and which would utilize the cortico-spinal pathway as a common output. The evidence for this hypothesis rests with the demonstration by micro-electrodes in the tremor monkey and in human Parkinson's disease of cells firing at the appropriate 3/8 sec. rate (8). These cells are found mainly in the posterior part of the ventro-lateral nucleus of the thalamus and in some-areas of the subthalamus. Other neighboring cells with rhythmic properties are also observed in this area, but they usually fire in response to a peripheral stimulus such as touch or movement of a joint.

It should be remembered that the ventro-lateral nucleus of the thalamus is also the area that must be destroyed for the best results in both parkinsonian and intention tremor syndromes. It is thus a reasonable proposal to assume for our discussion that what we will, somewhat clinically, call the »pulsating« cells of this nucleus are a necessary component of the »tremorigenic« system to analyse. It is also probable that, under ordinary circumstances, these cells function at an intensity insufficient to produce clinical symptoms. To create tremor would not require direct damage to this area, since this would eliminate the »pulsating« cells from the circuit, but rather one of three circumstances: triggering by an excitatory input from another center, removal of usually inhibitory dampening system, or both mechanisms working in series.

The available evidence indicates that the ventro-lateral nucleus of the thalamus receives inputs directly from the basal ganglia (mainly globus pallidus), the cerebellum (dentate nucleus) and indirectly from the reticular formation and perhaps the substantia nigra reticulata. At least two of these systems are facilitating, but even their output is carefully controlled and monitored by an inhibitory servo-mechanism. Thus the main output of the basal ganglia is through the globus pallidus to the VL, but this output is controlled by the inhibitory action of the dopaminergic circuit from the substantia nigra. A similar inhibitory mechanism (utilizing GABA as a probable neurotransmitter from the Purkinje cells) also monitors the dentato-rubro-thalamic output in the cerebellum. Finally it is well known from the studies of the Swedish group (9) that there exist parallel serotonergic and noradrenergic ascending pathways from the brain stem reticular formation to the thalamus. These pathways have been shown by Jouvet and others (10, 11) to be involved in sleep-waking mechanisms.

How these multiple incoming pathways interact at the level of the VL and how they activate the »pulsating« tremorigenic cells in this nucleus is the still unresolved problem. We have seen that most putative neurotransmitters, particularly acetylcholine, dopamine, serotonin, and noradrenaline are involved in some way in this mechanism, but their relationship and interaction is far from clear. At one time or another, most of these substances have been implicated in the mechanism of tremor. It is thus important for us to look closely at the evidence.

B — AMINES

a) Noradrenaline

Many years ago Schwab and his collaborators (12) obtained a marked increase in the tremor of Parkinson's disease patients after infusions of noradrenaline and adrenaline. This finding has been confirmed by others (13). It probably acts by the same unspecific mechanism as that postulated during fear or anxiety, where it is equally observed that all forms of tremor are enhanced. The studies of Andén et al. (9) have indicated that the ascending noradrenergic pathway from the brain stem reticular formation to the centre median and unspecific nuclei of the thalamus is probably involved in the alerting and awakening mechanisms (11). Such mechanisms would only indirectly contribute to stimulation of the VL »pulsating« cells by modifying the response threshold.

b) Dopamine

The best known tremor syndrome, of course, is Parkinson's disease, and in this illness the role of dopamine has been conclusively demonstrated. However a common misunderstanding has arisen which assumes that this amine is directly involved in the pathogenesis of all the symptoms of Parkinson's disease. That this is not completely so should be evident from a number of experiments and clinical observations:

- (a) the only established relationship between the urinary concentration of dopamine and a clinical symptom is with akinesia (16);
- (b) a specific decrease in dopamine produced by alpha-methyl-paratyrosine results only in hypokinesia in the animals so treated, unless previous damage had been inflicted to the brain stem area (17);
- (c) specific blocking of dopaminergic (adrenergic) receptors, such as with haloperidol results only in hypokinesia.

Thus the specific low striatal dopamine syndrome in man and animals is *hypokinesia*. This, of course, is also the symptom best influenced by L-DOPA treatment (4). The above observations, however, should not be taken to indicate that dopamine cannot be involved in the pathogenesis of tremor. It is known, for example, that L-DOPA can occasionally be very useful in alleviating parkinsonian tremor (4). It would appear that a dopamine deficiency is acting more as a facilitating factor to another mechanism. Nor is dopamine hyperactivity a likely culprit. L-DOPA overdose, in the presence of previous brain stem damage, has brought out chorea, tics or dystonia, but never the denovo production of tremor (4). Whenever tremor had persisted despite treatment, L-DOPA resulted in an increase in the amplitude of this movement with essentially no change in the basic rhythm. However in »essential intention tremor«, L-DOPA worsens the condition.

c) Acetylcholine

Although acetylcholine is the best known neurohormone and the only one for whom most of the criteria of a central transmitter substance have been met, there is yet no agreement as to its precise physiological function. Shute and Lewis (18) have shown that the previously postulated thalamo-cortical projections are at best indirect and through the capsula externa. Moreover Poirier and collaborators (19) have indicated that the most important cholinergic pathway may be the one from the striatum to the substantia nigra, thus completing a closed circuit with the dopaminergic nigrostriatal pathway. Such a possibility would be compatible with the experiments of Ernst (20) who produced gnawing in animals (a symptom usually associated with dopaminergic striatal stimulation) after implantation of eserine in the substantia nigra.

Stern and collaborators (21) and many others have shown that ACh infusion can cause tremor in animals. It is well known, of course, that standard anticholinergic medication will be useful in partially controlling the parkinsonian tremor. On the other hand they usually increase essential attitude tremor. However recent studies by Coyle and Snyder (22) indicate that these substances also affect dopaminergic systems and add complications to the accepted mechanism of action of anticholinergic drugs.

Although there is no doubt that acetylcholine is involved in tremorigenic mechanisms, it is not clear yet at what level it could be acting. The most likely mechanism is by saturating muscarinic receptors. In the short fiber integrating areas of the central nervous system, dopamine and acetylcholine appear to be playing the role assigned to noradrenaline and serotonin in longer fiber groups. Thus dopamine and acetylcholine should be considered the respective central modulators of the ergotrophic and tropotrophic nervous system and are always in precarious balance especially in integrating areas such as the striatum (27). The possible role of dopamine as a presynaptic inhibitor of ACh release strengthens this interrelationship.

d) Serotonin

This amine appears to be a likely candidate for playing a direct role in tremor production. It has been found in lower than normal concentration in the brain of parkinsonian patients (23) and in reserpine induced states where a tremor is frequently observed. It is highly concentrated in the brain stem, hypothalamus and limbic areas. It has been implicated in the regulation of sleep and of mood. Previous studies from our group (24) had shown that its synthesis is probably not normal in Parkinson's disease and that some parkinsonian tremors could be decreased with the precursor 5-hydroxytryptophan. According to Stern, the specific serotonin receptor blocking agent LSD-25 can also produce tremor (25), while 5-HTP can inhibit oxotremorine induced tremor. Moreover, Poirier and collaborators (26) have indicated that serotonin

may be an important neurotransmitter in the rubro-olivo-cerebello-rubral loop which they postulate is the essential physiological system in tremor.

Thus there is some evidence implicating many of the putative neurotransmitters in the production of tremor. Although the evidence is strongest for acetylcholine, it is impossible to disregard the others. Moreover there is now increasing agreement for important interactions between the various neurohormones, especially in centers where they are all found in high concentration, such as the striatum (27).

From the scattered facts reviewed above, can a useful pattern emerge? It is more physiologically useful to consider what happens at specific receptor sites, rather than to examine brain concentrations of any given amine, which are only imperfect reflections of a static state. With such a point of view, we could envision the *dynamics of tremor production* as involving:

- (1) understimulation of the *serotonin* receptors;
- (2) decreased stimulation of the *dopamine* receptors;
- (3) hyperstimulation of the *acetylcholine* receptors;
- (4) possibly also hyperstimulation of *noradrenergic* receptors.

The anatomic sites for each of these receptors are not yet clearly defined, and are probably multiple. However the above observations agree with the known interactions between neurotransmitters. Such interactions reveal a dopamine-acetylcholine antagonism in the striatum (24) as well as a serotonin-noradrenaline antagonism in the brain stem and spinal cord. Thus studies with Orzeck in our laboratory (29) have indeed shown that if low striatal concentrations of acetylcholine are produced in the rabbit by reserpine (with increased release), a return to normal levels (or release rate) can occur after giving L-DOPA or apomorphine to the animals, but not after 5-HTP. Conversely, Van Woert and co-workers have shown that the increased cholinergic sensitivity present in parkinsonian patients can be reversed by treatment with L-DOPA (30). The parallel pathways but opposing actions of serotonin and noradrenaline in the brain stem and spinal cord and their respective functions in the sleep-waking cycles have already been referred to (9, 10).

Thus under normal conditions the »pulsating« cells in the VL could be under a modulating influence of opposing serotonin and noradrenergic pathways from the brain stem, through the unspecific thalamic relays. In the presence of decreased serotonin stimulation (receptor block such as with LSD; damage to serotonergic pathways from the brain stem) the cells would become more susceptible to stimulation by noradrenaline.

Normally these »pulsating« cells maintain their tone through a modulating input from the basal ganglia. Such an input is usually sufficient to avoid excessive firing because of the filtering function of the striatum. In the presence of high cholinergic activity, such as that induced by a defect in the controlling dopaminergic pathway, the »pulsating« cells are activated and the force of their synchronized output is such that the appropriate cortical motor area is stimulated and clinical tremor results.

It should be emphasized that, to date, it is impossible to state which neurotransmitter actually modulates the »pulsating« cells from the striatum. We only know that part of this system is cholinergic.

Thus the biochemical pathophysiology of tremor cannot involve only a single system or neurotransmitter. The tremorigenic center in the thalamus is under regulating influences from the brain stem. Some of this mechanism probably involves output from the rubro-olivo-cerebellar-rubral loop of Poirier (26), from the substantia nigra reticulata and from the reticular formation. Serotonin may be an important neurohormone in these pathways. GABA, the cerebellar inhibitory transmitter could play a controlling role similar to that of dopamine in the striatum. Damage to the serotonergic pathways thus results in hyperreactivity of the »pulsating« VL cells.

Under normal conditions the output of the »pulsating« cells is kept in check by the restraining influence of the striatum. If the cholinergic component of this system loses its own controlling mechanism (the dopaminergic nigro-striatal system), the input from the basal ganglia overstimulates the VL cells. In the presence of a previous state of hyperreactivity of these cells, the bursts of firing necessary for clinical tremor are produced.

C — TRACE METALS

In the light of such a delicately balanced system, it is difficult to visualize what the various trace metals found in these regions are doing, and to what extent the production of tremor is mediated through them, as suggested by Prof. Stern (14, 15) from many pharmacological experiments. The trace metals are indeed known to be involved as co-enzymes in many metabolic functions and as necessary constituents of binding, release and transport mechanisms. It is thus unlikely that they normally undergo major abrupt changes. We would then like to postulate, in slight variance with Prof. Stern, that whenever a drug, or substance, causes a change in trace metals, it is not exerting its action directly through this effect, but through modifications in the underlying balance between neurotransmitters. This is true of course for chronic experiments. In acute toxicity metabolic cell damage may also play a role. Let us look at the evidence:

- 1) There is no doubt that the basal ganglia and mainly the globus pallidus and substantia nigra reticulata contain an abundance of trace metals. This is particularly striking in the case of iron.

- 2) A tremor similar to that of Parkinson's disease can be produced with tremorine and LSD-25, both substances which are said by Prof. Stern to lower brain iron. However both substances also act on amines (31).

- 3) A form of intention tremor, more like that seen in Wilson's disease, is produced by diethylcysteamine, penicillamine, thioacetamide or alpha-methyl-dopa (14, 15). These drugs are said by Prof. Stern to mobilize copper in the brain. Alpha-methyl-dopa, of course, also inhibits aminoacid decarboxylase which would result in a decreased formation

of serotonin. Using atomic absorption, we unfortunately have been unable to confirm changes in the brain copper concentration of mice after thioacetamide (Table 1), but have clearly demonstrated an increase

Table 1*
EFFECT OF THIOACETAMIDE ON BRAIN COPPER

DRUG	Cu (wet wt.)	Cu (dry wt.)
SALINE	3.61	17.51
THIOACETAMIDE	3.80	18.61

* Data from Donaldson, J.; Minnich, J., and Barbeau, A. (1971). in serotonin concentration (Table 2), in the same animals. With alpha-methyl-dopa, as with thioacetamide, it can be surmised that serotonin receptors were understimulated.

Table 2*
EFFECT OF THIOACETAMIDE ON BRAIN SEROTONIN
BRAIN CONTENT

	($\mu\text{g./gm.}$)	
(A) CONTROL ANIMALS (NaCl)	0.62	P
(B) THIOACETAMIDE (170 mg./kg. x 3 days)	0.72	> < 0.001
(C) NaCl + 5-H. T. P. (150 mg./kg.)	1.20	
(D) THIOACETAMIDE + 5-H. T. P.	1.93	> < 0.001

* Data from Donaldson, J.; Minnich, J., and Barbeau, A. (1971).

4) Costa and collaborators (32) have been able to produce in monkeys (with chronic manganese intoxication) a brain decrease in dopamine and serotonin and the clinical picture of akinesia and tremor.

5) Chronic injections of iron in rats cause a decrease in dopamine and serotonin and a rigid-tremulous symptomatology (33).

6) Chronic copper toxicity, such as in Wilson's disease, is apparently accompanied by increased striatal concentration of dopamine.

7) Finally, we have recently been able to obtain pure akinesia and a specific striatal dopamine deficiency in dogs treated with a magnesium deficient diet (17 ppm/days) for 35 days (34) (Table 3).

Table 3

CAUDATE AMINES AFTER A LOW MAGNESIUM DIET (Dogs — $\mu\text{g./gm. tissue}$; 6 animals/group) (mean $\pm$ S. E. M.)			
	DOPAMINE	NORADRENALINE	SEROTONIN
CONTROL ANIMALS	4.93 $\pm$ 0.47	0.072 $\pm$ 0.01	1.12 $\pm$ 0.11
LOW Mg ANIMALS	2.68 $\pm$ 0.39*	0.057 $\pm$ 0.04	1.10 $\pm$ 0.11

* p < 0.01 from control.

Thus trace metals may be important in the production of the symptomatology of extrapyramidal diseases, but only as contributing or trigger factors in induced changes in amine metabolism or through the production of subtle amine imbalances. In acute toxic experiments, such metals could probably also cause damage through anoxic or metabolic cell changes.

Thus like Prof. Stern, we feel that it is important to concentrate on a rigorous study of trace metals and ions in the pathogenesis of various diseases of the extrapyramidal system, but such studies should be of *chronically* induced minimal changes. Acute loading experiments probably will not contribute much to our understanding of these mechanisms. In this light we are presently studying the effect of chronic magnesium deficiency on the production of electrolyte and trace metal changes in the brain, on the concentration of enzymes and amines in different regions of the brain, and finally on the possibility of cell damage in specific areas. This model may serve for the study of Parkinson's disease. Similar models with copper, iron, manganese, potassium or calcium chronic loading or deficiencies, are also contemplated. The theoretical basis for the expected amine changes in such models cannot be reviewed in detail here, but is summarized in Table 4.

Table 4
AMINES AND EXTRAPYRAMIDAL DISEASES
(STATE OF RECEPTOR STIMULATION REQUESTED FOR POSSIBLE
MODELS OF SPECIFIC DISEASES)

DISEASE	DOPAMINE	SEROTONIN	ACETYLCHOLINE
PARKINSON	low	low	high
DYSTONIA	high	low	normal
ESSENTIAL	normal	low	low
TREMOR			
CHOREA	high	slightly high	low

SUMMARY

An hypothesis of the pathophysiology of tremor is presented: parkinsonian tremor is probably due to an increased synchronized output from normally »pulsating« cells in the ventro-lateral nucleus of the thalamus and parts of the subthalamus. The activation of these cells may originate from damage to incoming (probably serotonergic) pathways from the brain stem; the increased synchronized firing could result from removal of the dopaminergic filter mechanism in the striatum, with secondary hyperactivity of the cholinergic mechanism and hyperstimulation of cholinergic muscarinic receptors.

Such imbalance in multi-neurotransmitter systems may be the consequence of faulty enzymatic machinery. Defects in trace metals, or

electrolytes could be responsible for these mechanisms, but it is likely that such changes are produced slowly. Chronic loading, or deficiency, experiments have more chances of solving this equation than the almost toxic variations obtained during acute pharmacological manipulations. Theoretical models of the amine imbalance in various extrapyramidal disorders are given. Induced variations in trace metals or electrolytes should endeavour to produce such models clinically and biochemically. Preliminary results from a magnesium deficiency study are given.

A. BARBEAU, J. DONALDSON I J. L. MINNICH

ULOGA METALA U TRAGOVIMA KATEHOLAMINA U PRODUKCIJI TREMORA

KRATAK SADRŽAJ

Osim tremora u Parkinsonovoj bolesti, važnu tačku za kompletnu kliničku prezentaciju predstavljaju atitudinalni i intencionalni tremor u ostalim ekstrapiramidalnim poremećajima. Poznato je da će L-dopa doprinijeti redukciji Parkinsonovog tremora, dok će ga alfa-metil-dopa povećati; prava uloga serotonina još uvijek nije ispitana, jer se tremor može izazvati redukcijom ovog amina (Reserpin) u moždanom sadržaju ili visokim dozama prekursora 5-HTP. Tremorin i oksotremorin, koji prouzrokuju tremor, modificiraju moždani sadržaj dopamina, serotonina i acetilholina.

Isti paradoks postoji u eksperimentalno proizvedenom tremoru nakon egzogenih ili nutritivnih modifikacija tragova metala u moždanom sadržaju (željezo, mangan, bakar i magnezijum) ili nakon lijekova kao što su tremorin, karbon tetraklorid, tioacetamid, alfa-metil-dopa, L-dopa.

Prezentirana su ispitivanja na korelaciji između tragova metala i kateholamina u mozgu i pokušano je da se pokaže da simptom »tremor« rezultira iz kompleksne interakcije različitih važnih neurotransmitera i modulatora. U tim interakcijama relativna koncentracija različitih tragova metala (kao koenzimi i regulatori u sinaptičnoj membrani i transportu) može da bude »trigger« mehanizam.

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DISCUSSION

Bruggencate. I found your remark very interesting, that one needs a lesion within the rubro-dentato-rubro loop as an additional factor to produce tremor. As you certainly know, the organisation of midbrain-cerebellar systems is rather complex. Thus, N. ruber not only affects the inferior olive but also other cerebellopetal systems, for instance the VSCT. Lesion within the midbrain certainly can influence also this system. Don't you think that

it would be important to differentiate further between the various possibilities particularly since olive-cerebellar fibres are ending as climbing fibres, and the VSCT as mossy fibres? (And since nobody knows what these different fibres mean to the cerebellum).

Barbeau: I agree with you that the finite geography and histochemistry of this loop still has to be mapped out. I refer you to the excellent anatomical and physiological studies of Poirier and his collaborators who have been investigating the production of tremor after lesion of this loop in monkeys and cats.

Stern: I am very glad to see that prof. Barbeau found that Serotonin is important in the therapy of Parkinson's disease. Ten years ago in one of my works. I found together with colleague Gašparević that 5-Hydroxytryptofan and not L-DOPA abolishes the tremor of mice caused by tremorin.

Barbeau: Thank you prof. Stern. Another was to interfere with serotonin is to use LSD-25 which, as you know, also produces tremor.

Schnieden: 1) Could you clarify one point in your talk. You postulated that a decrease of 5-HT and dopamine and a rise in ACh is important in Parkinsonian tremor. Does this postulate apply only to slow resting tremor, or to both forms? 2) Could you comment on the pathophysiology of essential tremor?

Barbeau: Thank you for your questions. To the first question: We have mainly been considering Parkinsonian resting tremor when talking about the effects of a decreased dopamine stimulation upon the release of ACh and the saturation of the muscarinic receptors. The effect of the brain-stem serotonin pathway lesion is common to any sort of tremor: resting or intention (action). We would suspect that another servo-mechanism, similar to that using dopamine for the striatum, is operative in the cerebellum and we postulate that for intention tremor GABA plays the same controlling role as dopamine for rest tremor.

To the second question: I have no personal studies concerning the pathophysiology of essential tremor. I can only say that this form of tremor is made worse clinically by anticholinergic drugs as well as by L-DOPA. I would suspect that cholinergic receptor stimulation is decreased in the presence of possibly increased dopamine receptor stimulation.

Vizi: Have you got any idea of what is the role of magnesium level in Parkinson's disease? We have found that high Mg^{2+} (9.3 mM) is capable of inhibiting the ACh release induced by nicotine (5×10^{-6} g/ml) from the nerve terminals. Nicotine was used to mimic the pathological firing of neurone. Furthermore, it is generally accepted, that the actual concentration of Mg^{2+} can influence the ACh release. Could you tell us, whether during low Mg^{2+} level the probably increased ACh release is the factor which plays some role in producing of disturbances of extrapyramidal system?

Barbeau: Thank you for your remarks. We entirely agree that the most likely explanation for the action of Mg is upon the release mechanism of the neurotransmitter. However, the mode of action may be much more complex than we thought. During our Mg deficiency studies we also produce a decrease in serum Potassium concentrations. Since the diet was not deficient in Potassium, a shift towards intracellular pools is possible and may also have to be considered. It is too early to say how Mg deficiency may contribute to extrapyramidal system.

Gvörgey: You have mentioned the role of 5-HT in the parkinsonian tremor. Have you any data about the activity of methysergid or p-chlorophenylalanine: how they influence the function of extrapyramidal system or the drug-induced experimental tremor or rigidity?

Barbeau: We have no definite data on this point yet, but we should be reminded that p-chloro-phenylalanine is not specific to the brain-stem serotonin pathways. The limbic system, also rich in 5-HT, would also be affected. It is thus difficult to use such pharmacologic tools for the understanding of any given symptom, unless topical localized studies are carried out.

THE FUNCTION OF IRON IN THE FORMATION OF TREMOR*

Taylor et al showed recently that α - α dipyridil (D) when injected into rats inhibited tyrosine-hydroxylase (TH) in the suprarenal gland and that it reduces noradrenaline in the brain (41). The same authors noticed that rats which received D developed tremor.

This result interested us for many reasons. We have shown that oxotremorine (OT) significantly reduces the concentration of total iron (TFe) in the brain (17) and that the greatest reduction is in the corpus striatum (CS). We have also shown that the reduction of iron takes place in the mitochondria of the CS.

In addition to this we proved that the total flavine (TFI) in the complete brain of rats is reduced (20), as it is in the CS and the isolated mitochondria of the CS (40)

As the inhibition of TH prevents the production of DOPA, i. e. dopamine (46) and the function of this enzyme depends on iron (46, 26), the question is whether the tremor described by Taylor et al is a good model for Parkinson's disease, because it is known that the reduction of dopamine in the CS appears in the disease (25). Apart from that, L could give us a better comprehension of the role of iron at the beginning of Parkinson's tremor. So the question is: is the reduction of iron the cause or consequence of tremor.

It is known that TH is found in higher concentration in the nucleus niger (6) (NN). Therefore it was necessary to determine the iron in NN in tremor caused by D and OT. Apart from that, in this work we were interested in the influence of D on the quantity of acetylcholine (Ach) and dopamine in the brain of rat.

We also examined the influence of different drugs on tremor caused by D. We gave D locally in the globus pallidus and NN by means of stereotactic apparatus to see if the local reduction of iron in these two regions leads to tremor. As well as TFe we determined TFI and copper in the brain of rat because we have shown that tremorine reduces copper too (18). We also examined the effect of D on temperature, sense of pain and blood sugar.

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METHODS

Determination of iron in NN.

White rats of both sexes with a weight of 200—250 g. were used in the experiment. Oxotremorine (0,5 mg/kg) and D (25 mg/kg) were injected intravenously. 30 minutes after the administration of oxotremorine or D the animals were killed by anaesthesia with ether. The brain was washed out with normal saline in the following way: the thorax was opened, the abdominal blood vessels tied and the aorta ascendens in which a cannular was introduced for rinsing with normal saline was cut through. After that the vena cava was also cut through and the brain was rinsed until a clear fluid came out.

The brain was then taken out and after the removal of the small brain, it was cooled to -4°C . The cooled brain was isolated using the method of Popov et al (31). The NN, which is in region 8 (Tegmentum), is clearly differentiated from the rest of the brain mass and by using a magnifying glass with a fine occulistic scalple everything except the NN in which Fe was determined, was removed so that the tissue was homogenous, and the iron was determined spectrometrically (7). TFI and copper in CNS we determined by means of Bessey's (3) method in addition to Heilmeyer et al (21).

Statistical analysis of the results was done by the method of sequential analysis.

Dopamine was determined spectrofluometrically with Carlson and Waldeck's method (5). Ach was extracted by McIntosh and Perrys method (26) and the rectus abdominalis of frog was measured.

Of drugs we gave atropine sulphate, caramiphen, ethyl benztropine, penicillamine and alfa-methyl dopa, which inhibit intentional tremor (38), alfa-methyl-meta-tyrozone a depletor of dopamine (47), gamma-hydroxybutyrate which increases dopamine in CNS (10), and DL-4-chloramphetamine a depletor of serotonin (9). In addition we determined the activity of cholinesterase in vitro and in vivo by Michelson's method (27), 30, 60, and 180 mins after giving D in the brain and erythrocytes.

By means of steriotactic apparatus (4), using Groot's atlas (14), we injected D in a dose of 1 or 10 gamma (in 2 mm) by means of »Agla« syringe directly into the globus pallidus, nucleus caudatus and nucleus niger and observed if tremor developed. The next day the animals were killed, the brains fixed in formalin and microscopic control of the position of the needle of the steriotactic apparatus was carried out. We measured tremor in the oculus which was observed always by the same technical assistant and two assistants who did not know the groups gave the estimation of tremor and compared it with that given by the technical assistant.

Because a static tremor caused by OT causes analgesis (8) and reduction of temperature (8) as well as an increase of blood sugar (16) we also examined analgesia by means of a clamp on the tail. Control rats in 3—4 secs. tried to remove the clamp with their lips, became agitated and revolved. In the analgezic effect, the trying to remove the clamp developed later or not at all, and the animals were more peaceful. We also determined the rectal temperature and the blood sugar level using the method of Gubler et al. (15) after giving D.

RESULTS

First of all we must emphasize that we noticed D (100 mg/kg i. p.) causes static tremor as well as OT. This tremor only appears after 30—60 mins and lasts 6—7 hours and sometimes longer. Any way, much longer than OT tremor.

Table I

THE EFFECT OF D (100 mg/kg i. p.) ON TFe, TFl AND Cu In THE BRAIN OF RAT IN γ/g

(8 animals in each group)

E X P E R I M E N T S

	Control	30'		60'		90'		120'	
TFe	82,6	60,3	$p < 0,005$	70,7	$p < 0,005$	86,3	$p < 0,01$	96,8	$p < 0,005$
TFl	2,430	2,513	n. s.	1,833	$p < 0,005$	2,071	$p < 0,005$	2,360	n. s.
Cu	4,30	2,00	$p < 0,005$	2,42	$p < 0,005$	3,51	$p < 0,005$	4,40	n. s.

Values are expressed as γ/g tissue.

Subst.: α , α' — dipyrldyl

Dose: 100 mg/kg i. p.

Tissue: brain

As we can see from table I, D leads to a significant, reduction of the concentration TFe after 30, 60, 90, 120 mins., TFl is significantly reduced after 60 and 90 mins., and Cu after 30, 60 and 90 mins. D signi-

Table II

TFe IN SUBSTANTIA NIGRA OF RAT'S BRAIN TREATED PREVIOUSLY WITH $\alpha\alpha$ DIPYRIDYL* AND OXOTREMORINE

Control	D		OT	
96,95	66,86	$p < 0,005$	88,07	$p < 0,005$
C	D ₁	p C:D ₁	D ₂	p C:D ₂

* Dipyrldyl 100 mg/kg i. p.; OT 0,25 mg/kg i. v.; the animals were killed 30 min. after application of drugs.

Table III

THE EFFECT OF DIPYRIDYL (100 mg/kg i. p.) ON THE LEVEL OF TOTAL ACETYLCHOLINE IN THE BRAIN OF RAT

Dipyrldyl was given i. p. (100 mg/kg). The values are expressed as γ/gr fresh tissue.

N. S. is not significant.

	No of animals	Acetylcholine	change in % of control values	p
Control	7	2,65 $\pm$ 0,08	—	—
1h	4	3,13 $\pm$ 0,18	+18	<0,01
2h	6	3,30 $\pm$ 0,09	+24	<0,001
3h	5	3,48 $\pm$ 0,14	+31	<0,001
4h	4	3,22 $\pm$ 0,11	+21	<0,01
12h	6	2,74 $\pm$ 0,08	+ 3	N. S.
24h	4	2,67 $\pm$ 0,17	+ 1	N. S.

Table IV

SUBSTANCE	Time of application of substance	Dosis mg/kg	Effect
Atropine sulfat	15	10 i. p.	++
"	15	20 i. p.	—
Methylatropine nitrate	15	20 i. p.	++
Caramiphen	15	10 i. p.	++
"	15	10 i. p.	—
Ethylbenztropine	15	1 i. p.	—
Penicillamine	15	50 i. p.	+++
α -methyl-dopa	4h	100 i. p.	+++
α -methyl-meta-tyrosin	4h	100 i. p.	++
DL-4-chlor-amphetamin	4h	10 i. v.	++
γ -hydroxybutirat	60'	500 i. v.	++
L-Dopa	60'	100 i. v.	++
5-hydroxytryptophane	60'	100 i. p.	++
Control	—	—	++

ificantly reduces TFe in the NN of rat (Table II). From table III we see that D significantly increases ACh 2, 3 and 4 hours after application. After 24 hours ACh returns to normal.

From table IV it can be seen that known cholinolytic-antiparkinsonics (atropine, caramiphen, ethylbenztropine) removed static tremor caused by D. Substances which inhibit intentional tremor of Wilson's type (penicillamine, α -methyl-dopa) (38) even increase this tremor. α -methyl-meta-tyrosine, DL-4-chloramphetamine, gamma-hydroxybutyrate, L-Dopa, and 5-hydroxytryptophane, are without effect.

D is without effect on the activity of cholinesterase in erythrocytes and brain of rat after 30, 60 and 180 mins.*

From table VI we see that the application of D leads to a reduction of temperature as well as to analgesia and an increase of the blood sugar.

Table VI

THE AMOUNT OF DOPAMINE $\mu\text{g/g}$ IN FRESH TISSUE
OF RAT BRAIN TREATED PREVIOUSLY BY D

Substance and dose	ug DOPAMINE / g tissue		
	Control	4h	24h
D 100 mg/kg i. p.	0,60 ($\pm 0,12-6$)	0,60 ($\pm 0,12-6$)	0,51 (0,11—12) p < 0,01

From table VI we see that dopamine is reduced after 24 hours.

The injection of D directly into specific regions of the CNS led to expressed static tremor only when we gave 10 gamma and only in the nucleus niger.

* Our thank a due too Dr. B. Bošković (ITMZ, Beograd) who measured the activity of cholinesterase.

Table V

THE EFFECT OF α , α' DIPYRIDYL (100 mg/kg i. p.) ON RAT BODY TEMPERATURE, PAIN AND BLOOD SUGAR OF THE RAT

a) Rat body temperature

Body temperature of control animals	Body temperature after the application of α , α' dipyridyl						p
	2 hours	2.5 hours	3 hours	4 hours	24 hours		
1	2	3	4	5	6	7	
37,86 $\pm$ 0,28(6)	33,33 $\pm$ 0,36(6)	32,83 $\pm$ 1,01(6)	32,4 $\pm$ 0,65(5)	32,68 $\pm$ 0,52(5)	36,92 $\pm$ 0,42(5)		1:2 < 0,01 1:3 < 0,01 1:4 < 0,01 1:5 < 0,01 1:6 > 0,05



b) Pain

Reaction to pain before the application of α , α' -dipyridyl	Reaction to pain 2 hours after the application of α , α' -dipyridyl	p
2,10 $\pm$ 0,77(9)	5,16 $\pm$ 0,75(9)	1:2 < 0,05

c) Blood sugar

Blood sugar concentration of control animals	Blood sugar concentration after the application of α , α' -dipyridyl			p
	2h	4h	24 h	
122 $\pm$ 4,22(6)	158 $\pm$ 10,61(6)	162 $\pm$ 12,39(6)	120 $\pm$ 10,17(5)	1:2 < 0,05 1:3 < 0,05 1:4 > 0,05

All numbers in the table represent the mean values $\pm$ of standard error
The numbers in brackets represent the number of treated animals



DISCUSSION

As we see D causes static tremor surprisingly similar to tremor caused by OT in its biochemical effects. There is an increase in ACh in CNS as well as with OT (22, 30). This tremor can be inhibited with known anti-parkinsonic agents, although slightly higher doses are needed for this than in tremor caused by OT, except for ethyl-benzotropine which is known to act entirely centrally (45). D as well as OT (22) does not inhibit cholinesterase which we think is very interesting, because it follows from this that the increase of ACh is produced because of some other reasons or because of the activation of cholineacetylase, which we have already found for OT (32). It is also interesting that D leads to a reduction in TFe, TFl and copper, which is known also for OT (39, 40, 20, 18). Tremor caused by D also reduced temperature and blood sugar, and caused analgesia, the same as OT. We see that substances which reduce or increase dopamine and serotonin in CNS are without effect on D tremor, and we see a similarity of action of D and OT. We see that D changes the quantity of dopamine in the brain of rat after 24 hours.

It is very interesting that injection of D only in the nucleus niger leads to tremor. We emphasise this especially because it is known that the region contains most TH (6). We have said that this enzyme is dependant on iron (46, 26) and it is known that D combines with iron. In connection with this it is interesting to mention that we showed earlier that tripyridine, which combines with iron too (29), also causes forced movements in mice similar to chorea (37). We showed earlier that deferoxamine, a substance which quite specifically combines with iron (45), if given intracranially to mouse, also causes static tremor (45). Accordingly the question is, what is the cause of static tremor if we compare D and OT. We see that both substances lead to the same biochemical changes in CNS (increase of ACh, fall of TFe, TFl, and action of cholinesterase unchanged). On the other hand we showed that a continuous infusion of ACh in globus pallidus also causes static tremor (35).

We think that from all this it is possible to say that the reduction of iron is of primary importance in the formation of experimental tremor with either OT or D. With this it is necessary to emphasise that the globus pallidus and the nucleus niger are the richest regions in iron in the CNS (44, 32). The decrease in iron in those regions leads to an increase of ACh. We still do not know if it is because of the liberation of ACh from some depot or because of the activation of cholinacetylase. We also do not know the role of TFl.

In this observation it is significant that D as well as OT leads to a reduction of iron, that they do not inhibit cholinesterase and that they increase significantly ACh in CNS. However an infusion of ACh directly into the globus pallidus also causes static tremor (35). In accordance with all of these findings it looks as if an increase of ACh in CNS, which is the real cause of tremor, leads to a reduction of iron. That ACh is the direct provocator of static tremor is seen after the application of chemicholine-3 directly into the globus pallidus which prevents static tremor caused by OT (34, 36).

We should emphasise that in patients with Parkinson's disease, there is an increase of elimination of iron in the urine if they are given an injection of deferoxamine (2).

We found that Cu^{++} is reduced after giving D as after OT (18). Cu is essential for the function of dopamine-beta-hydroxylase (11). Maybe because of this a reduction of dopamine is not found in acute experiments as on one hand TH increases its formation and the inactivation of dopamine- β -hydroxylase prevents its degradation.

Recently Bapta et al showed that D does not lead to inhibition of tyrosine-hydroxylase (1). According to them dopamine and serotonin do not change and the transformation of radioactive tyrosine in dopamine and tryptophan in serotonin develops normally if animals receive D (1). Our results are not quite the same because we found that dopamine was significantly reduced in the brain of rat, 24 hours after D was given. Besides this Bapta et al (1) could not remove rest tremor produced by D, with atropine, but we succeeded not only with atropine but with other antiparkinsonics too. On examining our findings, Taylor et al. (42), and those of Bapta et al. (1), it looks as if the reduction of iron is of essential importance, because we got the same results with an injection of deferoxamine in the globus pallidus (37). According to this, all that reduces iron in the CNS leads to static tremor which can be inhibited with antiparkinsonic substances. We did not measure the activity of tyrosine hydroxylase, and we cannot say if Taylor et al (42) or Bapta et al (1) is right. The fact is that the activity of tyrosine hydroxylase after D can be reactivated with the addition of Fe^{++} (42) According to our lastest experiments, an intracerebral injection of deferoxamine increased ACh in CNS too, which is also how oxotremorine and D work. Because of this it looks as if the reduction of iron leads to increased activity of cholineacetylase, which is already proved for tremorine (33) and recently als for OT (23). We cannot say mow if there is a reduction of tyrosine hydroxylase at the same time, but it seems that it is not essential as oxotremorine causes rest tremor but dopamine is certainly not reduced. It is difficult to say if the reduction of iron because of D, is important for the eventual inactivation of dopamine- beta-hydroxylase in vivo, because we found that copper decreases after D is given. It is already known that in vitro D reduces the activity of dopamine-beta-hydroxylase (12, 13).

Finally we would like to say that Voroshilovskaya and Reitses (48) found that adrenalectomy increased iron in CNS. We could indeed show that in adrenalectomised rats, tremor caused by oxotremorine and D either does not develop or is exceptionally weak in comparison with controls.

We should like to quote experiments with deoxyfrenolicine. This antibiotic inhibits TH in vitro and in vivo (43) However the inhibition cannot be removed with Fe^{++} in comparison to D. Direct injection of deoxyfrenolicine into the NN (10 gamma) did not cause tremor. We think that this also speaks for our hypothesis that a reduction of Fe in CNS is clear in certain regions, and is essential for the development of Parkinsonic tremor.

CONCLUSION

α - α -dipyridyl as well as oxotremorine, causes static tremor which can be removed by means of anticholinergics. This substance as well as oxotremorine usually causes biochemical changes, reduction of total iron and total flavines, and increases acetylcholine in the brain of rat. Further it reduces temperature, acts analgetically and increases the blood-sugar in the same way as oxotremorine. Taylor et al found that α - α -dipyridyl inhibits tyrosine hydroxylase while Bapta et al could not prove this.

On the basis of our own experiments it is concluded that for the existence of tremor caused by α - α -dipyridyl, oxotremorine or deferoxamine, the reduction of iron in the CNS is essential but the inhibition of tyrosine hydroxylase is not.

P. ŠTERN, E. HASANAGIĆ I R. IGIC

ULOGA ŽELJEZA U IZAZIVANJU TREMORA

KRATAK SADRŽAJ

Budući da tirosin-hidroksilaza može vršiti svoju aktivnost samo u prisutnosti željeza, a ona je jedan od faktora u stvaranju dopamina, autori su ispitivali djelovanje alfa-alfa-dipiridila, jedne supstance koja veže na sebe željezo u odnosu na statički tremor. Pokazalo se je da alfa-alfa-dipiridil izaziva statički tremor istog tipa kao i oxotremorin, s istim biokemijskim promjenama i drugim manifestacijama koje su vezane uz djelovanje oxotremorina. Tako npr. alfa-alfa-dipiridil također snizuje ne samo željezo, nego i bakar i ukupne flavine u mozgu štakora. Ova supstanca dovodi i do pada temperature, zatim smanjuje osjet bola kao i oxotremorin. Budući da injekcija ove supstance u nucleus niger dovodi do statičkog tremora, a to isto uzrokuje i injekcija deferoxamina, zaključuje se da je željezo bitno za postanak tremora i da njegov pad uzrokuje porast acetilholina u mozgu. Sve ove vrste tremora imaju i tu zajedničku karakteristiku da se mogu inhibirati antiholinergicima.

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RISTO BOKONJIC

TRACE METALS PATTERNS IN SOME EXTRAPYRAMIDAL DISEASES

The content of different trace metals in normal and pathologic human brain tissue has been investigated a decades ago by different techniques (Bodansky 1922, Tingey 1937, Alexander and Myerson 1938, Butt et al. 1954, 1958 and others) but the role o these findings are not completely understood.

After the famous findings of Cumings 1948 on excessive amount of copper in brain tissue from persons died of Wilson's disease, the attention was paid to other diseases and other related and non related trace metals.

Gumings mentioned a slight increase of iron (Fe) in Wilson's disease and Butt and coowork. increase of silver (Ag) content in whole brain. Lowental 1958 only noticed the increase of aluminium (Al) in brain tissue of Wilson's disease.

Huntington's chorea, another degenerative, dominantly inherited extrapyramidal disease, characterized by choreatic jerks, was rarely object of trace metal investigations in brain tissue. The only paper dealing with brain's trace metals in this disease was published by Courville and coowork 1963. They found decrease of iron and copper in almost all investigated regions, but significant increase of Strontium (Sr) in brain.

The task of this work was to prove the content of trace metals (iron, copper, aluminium, silver) in brain tissue from persons died of Huntington's chorea and Wilson's disease by very sensitive technique-emission spectrography.

MATERIAL AND METHOD

10 brains were used from persons died from Wilson's disease and 7 from those of Huntington's chorea. The control group consisted the brain tissue from healthy people killed in accident.

All brain tissue was previously formoled, but Lowental 1958 showed that formolisation of material did not essentially change the content of trace metals in the tissue.

In Wilson's disease determinations were made in brain cortex, white matter, basal ganglia, cerebellum, brain stem and spinal cord. In Huntington's chorea only brain cortex, white matter and basal ganglia were examined on trace metals content.

Tissue was cut with plastic instruments which were chemically cleaned in order to avoid possible contamination. All laboratory vessels built of quartz, were chemically cleaned, too.

The tissue was dried during 72—90 hours and after that put in Kjeldahl's vessel for mineralisation by wet way, using nitric acid (D 1, 39) and perchloric acid (D 1, 61) for analysis, and continuous heating. The mineralisation was made until complete destruction of organic components i. e. complete decolorisation of vessel's content. This procedure lasted several days.

Mineralized content of vessel was mixed with sodium carbonate and germanium dioxide (relation 150:1) and homogenised pastilles were made.

Spectrographic analyses were performed on a spectrograph with quartz optic and focal distance of 60 cm. Excitation was gained from the Durr generator giving an alternative arch of 50 periods per second, at 36 micro F, 10 Ohms. 800 micro Hz. Precombustion lasted 5 seconds and combustion 30 seconds.

Analytical factor (ratio between known quantity of trace metal and germanium) was read on spectroprojector Zeiss and diagramme of dosage (DD) was used for interpretation of spectra and computation of trace metal expressed in mg/100 g of dried tissue matter.

RESULTS

The results of these determinations, including the quantity of wet matter (WM), dried matter (DM), as well as percentage of later with computed standard error of the mean are presented on following tables:

Table I

Region	No of determinations	WM	DM	%	Fe _d	± St. Er.
Cerebral cortex	8	3,8704	0,9451	28,2	43,6	3,20
Subcortical ganglia	10	3,3365	0,7123	20,4	54,7	2,70
White matter	10	3,8921	0,9782	24,5	33,5	2,30
Cerebellar cortex	8	5,3662	0,8127	16,5	53,0	1,90
Cerebellar white matter	7	1,9148	0,4740	24,8	49,0	2,75
Brain stem	7	1,6643	0,5169	31,3	57,0	3,25
Spinal cord	7	1,0264	0,2719	26,1	55,4	2,40

Table I showing the content of Fe in different regions of brain in Wilson's disease

Table II

Region	No of determinations	WM	DM	%	Cu	± St. Er.
Cerebral cortex	8	3,8704	0,9451	28,2	24,7	1,45
Subcortical ganglia	10	3,3365	0,7123	20,4	36,0	1,70
White matter	10	3,8921	0,9782	24,9	29,9	0,95
Cerebellar cortex	7	5,3462	0,8127	16,6	35,0	1,90
Cerebellar white matter	7	1,9148	0,4740	24,8	35,5	1,50
Brain stem	7	1,6643	0,5169	31,3	35,6	1,60
Spinal cord	7	1,0264	0,2719	34,7	14,7	1,05

Table II showing the content of Cu in different regions of brain in Wilson's disease.

Table III

Region	No of determinations	WM	DM	%	Al	± St. Er.
Cerebral cortex	8	4,0848	1,4277	32,1	52,0	4,07
White matter	10	4,5152	1,0282	25,1	20,9	3,7
Subcortical ganglia	10	3,3156	0,7002	19,0	14,3	1,2
Cerebellar cortex	6	5,0065	0,8306	17,6	10,5	0,67
Cerebellar white matter	6	1,0750	0,4472	25,1	8,7	1,0
Brain stem	6	1,6643	0,5169	31,3	27,3	1,9
Spinal cord	6	1,0364	0,2719	26,1	40,3	0,95

Table III showing the content of Al in different regions of brain in Wilson's disease.

Table IV

Region	No of determinations	WM	DM	%	Ag	± St. Er.
Cerebral cortex	8	4,0848	1,4277	32,1	1,1	0,2
White matter	10	4,5152	1,0282	25,1	0,52	0,1
Subcortical ganglia	10	3,3156	0,7002	19,0	0,41	0,05
Cerebellar cortex	6	5,0065	0,8306	17,6	2,1	0,3
Cerebellar white matter	6	1,0750	0,4472	25,1	0,65	0,1
Brain stem	6	1,6643	0,5169	31,3	2,1	0,9
Spinal cord	6	1,0364	0,2719	26,1	2,1	0,07

Table IV showing the content of Ag in different regions of brain in Wilson's disease.

Table V

Region	No of determinations	WM	DM	%	Fe	± St. Er.
Grey matter	7	1,7744	0,2948	16,3	17,95	2,39
White matter	7	1,9768	0,5519	27,4	10,78	0,97
Basal Ganglia	7	1,2627	0,2496	17,9	16,85	2,07

Table V showing the content of Fe in different regions of brain in Huntington's chorea.

Table VI

Region	No of determinations	WM	DM	%	Cu	± St. Er.
Grey matter	7	1,7744	0,2948	16,3	2,35	0,36
White matter	7	1,9768	0,5519	27,4	1,61	0,11
Basal Ganglia	7	1,2627	0,2496	17,9	2,18	0,18

Table VI showing the content of Cu in different regions of brain in Huntington's chorea.

Table VII

Region	No of determinations	WM	DM	%	Al	± St. Er.
Grey matter	7	1,7744	0,2948	16,3	16,07	2,50
White matter	7	1,9768	0,5519	27,4	11,7	1,09
Basal Ganglia	7	1,2627	0,2496	17,9	12,80	2,17

Table VII showing the content of Al in different regions of brain in Huntington's chorea.

Table VIII

Region	No of determinations	WM	DM	%	Ag	± St. Er.
Grey matter	6	1,7744	0,2948	16,6	7,81	3,06
White matter	7	1,9768	0,5519	27,4	2,52	0,50
Basal Ganglia	7	1,2627	0,2496	17,9	4,77	1,04

Table VIII showing the content of Ag in different regions of brain in Huntington's chorea.

COMMENT

The content of iron in all examined regions of brain in Wilson's disease confirms the results of Cumings (1948) but the increase of this trace metal is not significant except in spinal cord.

Well known fact of significant, about 10 fold increase of copper content of whole brain, revealed by Cumings (1948), is confirmed by this method of determination (see Table III).

Aluminium, trace metal, so far little examined is significantly increased in all examined regions in Wilson's disease, paralel with those of copper. This fact was only mentioned by Lowental (1958) but not completely elaborated.

We could not confirme the findings of Butt and coowork. (1958) concerning increase of silver content of brain tissue in Wilson's disease. On the contrary, the content of silver is significantly decreased in regions of pronounced pathologic process, i. e. gray matter, white matter, and basal ganglia.

As far as Huntington's chorea is concerned, the profil of trace metals content show some difference. The data of Courville and coowork. (1963) regarding moderate decrease of iron and copper content in brain cortex, white matter and basal ganglia were confirmed by this technique. These decreases are highly significant. ($p < 0,0001$). We tried to prove the findings of same investigators concerning the increase of Strontium (Sr) in brain, but the content of this metal in brain tissue of Huntington's chorea, as well as in control group, was so small that could not be determined by this technique.

The principal finding is a highly significant ($p < 0.001$) increase of Aluminium (Al) in all examined regions, the fact not revealed so far.

Possible implications of these findings are numerous the changes in trace metals content might be related to proliferation of blood vessels or glia cells, changes of blood brain barrier system, changes in nerve cell components, enzyme systems and others. The aim of these investigations is solely to be a contribution to chemical pathology of the nervous system.

SUMMARY

The author presents the results of determination of some trace metals in different regions of human brain from persons died of Wilson's disease and Huntington's chorea. Determinations were made by emission spectrography after drying the tissue and destroying organic components. Iron (Fe), Copper (Cu), Aluminium (Al) and Silver (Ag) were determined in brain cortex, white matter basal ganglia and some other regions of brain.

Beside of very well known fact of extreme increase of copper content in brain tissue from patients died of Wilson's disease, significant increase of Aluminium and decrease of Silver has been found. Previous

findings of significant increase of Strontium in brain tissue in Huntington's chorea were not confirmed, but highly significant decrease of Iron and Copper as well as increase of Aluminium was found.

RISTO BOKONJIC

SADRŽAJ NEKIH METALA U MOŽDANOM TKIVU PRILIKOM EKSTRAPIRAMIDALNIH OBOLJENJA

KRATAK SADRŽAJ

Izloženi su rezultati određivanja oligoelemenata u moždanom tkivu kod pacijenata umrlih od Wilsonove bolesti i Huntingtonove horeje. Determinacije su vršene u formolisanom tkivu poslije prethodnog sušenja metodom emisije spektrografije. Redovno su determinacije vršene u moždanoj kori (CC), supkortikalnim ganglijama (BG) i u bijeloj masi (SA).

Prilikom Wilsonove bolesti, pored dobro poznatog povećanog sadržaja bakra (Cu) u svim regijama, nađeno je i signifikantno povišenje sadržaja aluminijuma (Al) u svim ispitivanim regijama. Pored toga, nađeno je i signifikantno sniženje sadržaja srebra (Ag).

Prilikom Huntingtonove horeje je nađeno signifikantno sniženje sadržaja gvožđa (Fe) i bakra (Cu) u svim ispitivanim regijama. Sadržaj aluminijuma (Al) visoko je signifikantno povišen u svim ispitivanim regijama. Ranije nađeni povećan sadržaj stroncijuma (Sr) prilikom ovog oboljenja nije mogao biti potvrđen.

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DISCUSSION

Dimitrijević: One synic comment. What you call by normal human brain in neurochemistry.

Bokonjić: Brain tissue of normal man which did not show any abnormality, and taken from healthy people killed in accident.

Schnieden: Could I have some information on the physiological role of aluminium?

Bokonjić: The physiological role of aluminium is little examined. I suspect Al is important trace metal in metallo-enzymes complex of LDH. In somewhere, especially in liver, that is very well known. LDH activity in brain has been examined by Dr Richard and some paralelism has been found.

Barbeau: I may not agree that using formaline treated brains is safe when studying trace metals. Using fresh materials is preferable because our experience is that formalin does contain contaminans. This may not explain the large changes in Cu or Al that you found, but would be expected in Parkinson's disease.

Bokonjić: Formalisation of material does not change so much the content of trace metals in gross neurochemistry. Comparison of trace metal content from fresh brain and formalised brain showed little, not significant differences.

A. O. ŽUPANČIČ

**A MODEL OF CHOLINORECEPTORS
BASED ON CONFORMATIONAL TRANSITIONS OF
MEMBRANE-BOUND ACETYL-CHOLINESTERASE PROTEIN**

1. INTRODUCTION

According to modern views, receptors can be defined as allosteric proteins of effector membranes which, under the influence of activators, undergo a conformational transition triggering herewith the chain of effects. Such conformational transitions cannot be directly proven, but can be inferred from the kinetics of reactions between active substances and the receptor protein. The working hypothesis on the identity of anionic sites of membrane-bound acetyl-cholinesterases (acetyl-ChE) with cholinoreceptor (ChR) sites (1, 3, 7, 8, 9) predicts a kinetic behaviour of this enzyme analogous to that of ChR; it should be noted that this hypothesis does not link ChR with the esteratic sites of acetyl-ChE, but with the anionic ones, which might be located on the neighbour polypeptide chain of the same macromolecule (4, 5). From this point of view, the kinetics of acetyl-ChE built-in into mouse diaphragm motor endplates was studied under the influence of cholinergic activating or blocking agents, and departures from the Michaelis-Menten kinetics were interpreted in terms of conformational transitions of acetyl-ChE protein (10, 11). Such kinetic experiments were later extended: a) to some more substances, and b) to acetyl-ChE of the frog heart ventricle, i. e., to a tissue responding to acetylcholine (Ach) with the muscarinic effect.

2. METHODS

Acetyl-ChE, built-in into either mouse diaphragm endplates or into the frog heart ventricle, was used as enzyme preparation; its activity was measured by continuous pH-static titration. The technical details were the same as described previously (11), except that Tris — HCl was omitted, and that in experiments with the frog ventricle acetyl-ChE, the NaCl concentration of the reaction mixture was lowered to 6.5 g/l.

3. RESULTS

The main results are summarised in Table 1.

Table 1.

TYPE OF KINETICS OF TWO MEMBRANE-BOUND AND ONE PURIFIED ACETYLCHOLINESTERASE (ACETYL-ChE) PREPARATIONS

Enzyme preparation	Compounds	Michaelis-Menten kinetics	n_H
acetyl-ChE of motor endplates	Ach (8 μ M — 0.25 mM)	obeyed	1
	Mch (63 μ M — 1 mM)	not obeyed	2
	Ach + TC (1 μ M)	not obeyed	2
	Ach + Gal (2.5 μ M)	not obeyed	2
	Ach + TEA (1 mM)	not obeyed	2
	Ach + TMA (3 mM)	obeyed	1
	Ach + Amb (2 nM)	obeyed	1
	Ach + E (0.1 μ M)	obeyed	1
	Ach + WIN ₇₇₃ (0.1 μ M)	not obeyed	2
	Ach + WIN ₃₃₁₇ (0.1 μ M)	obeyed	1
	Ach + Atr (1 μ M)	obeyed	1
acetyl-ChE purified (Worthington)	Ach (8 μ M — 0.25 mM)	obeyed	1
	Ach + TC (1 μ M)	obeyed	1
	Mch (63 μ M — 1 mM)	obeyed	1
acetyl-ChE of frog heart ventricles	Ach (8 μ M — 0.25 mM)	obeyed	1
	Ach + Atr (0.1 μ M)	not obeyed	0.5

Ach: acetylcholine. Mch: acetyl- β -methylcholine. TC: d (+) tubocurarine. Gal: gallamine. Amb: ambenonium. TEA: tetraethylammonium. TMA: tetramethylammonium. E: eserine. WIN₇₇₃ and WIN₃₃₁₇: for their chemical structures see (6), Atr: atropine. n_H : the Hill coefficient at substrate concentrations lower than about 0.1 mM.

4. DISCUSSION

Our membrane-bound acetyl-ChE preparations are mixtures of various proteins and other molecules; even so the binding of the investigated substances to other molecules than acetyl-ChE is irrelevant since acetyl-ChE activity is the only measured parameter.

Deviations from the Michaelis-Menten kinetics, as listed in Table 1, refer only to the membrane-bound acetyl-ChE while the purified enzyme always obeyed the usual kinetics.

With the endplate acetyl-ChE some substances change the Michaelis-Menten kinetics in the sense that the Hill coefficient (n_H) increases from ~ 1 to ~ 2 ; in other words, the rectangular hyperbola in the activity-substrate concentration plot changes to an S-shaped curve (11). Interpreting the responses of nicotinic ChR, Changeux and Podleski (2) pointed out that a hyperbolic dose-response curve indicates that the affinity of individual subunits for the ligand is independent of whether the neighbour subunits are occupied by the ligand or not ($n_H \sim 1$); an S-shaped curve, on the other hand, is indicative of a conformation where

the binding of the ligand to one subunit enhances the affinity of still unoccupied neighbour subunits for the ligand ($n_H \approx 2$).

From the first section of Table 1 it can be seen that five out of eleven tested substances cause a change from the hyperbolic ($n_H \sim 1$) to the S-shaped kinetic type ($n_H \sim 2$). In the concentrations used, the same five substances are cholinergic blocking agents, while the remaining six are either cholinergic activators or indifferent to nicotinic ChR. One is tempted to call this relation a rule, especially since such analogue pairs as Ach and Mch, TMA and TEA, WIN₃₃₁₇ and WIN₇₇₅ are included: in spite of the close structural similarity between the two members of each pair, they influence both the kinetics of acetyl-ChE and that of nicotinic ChR differently.

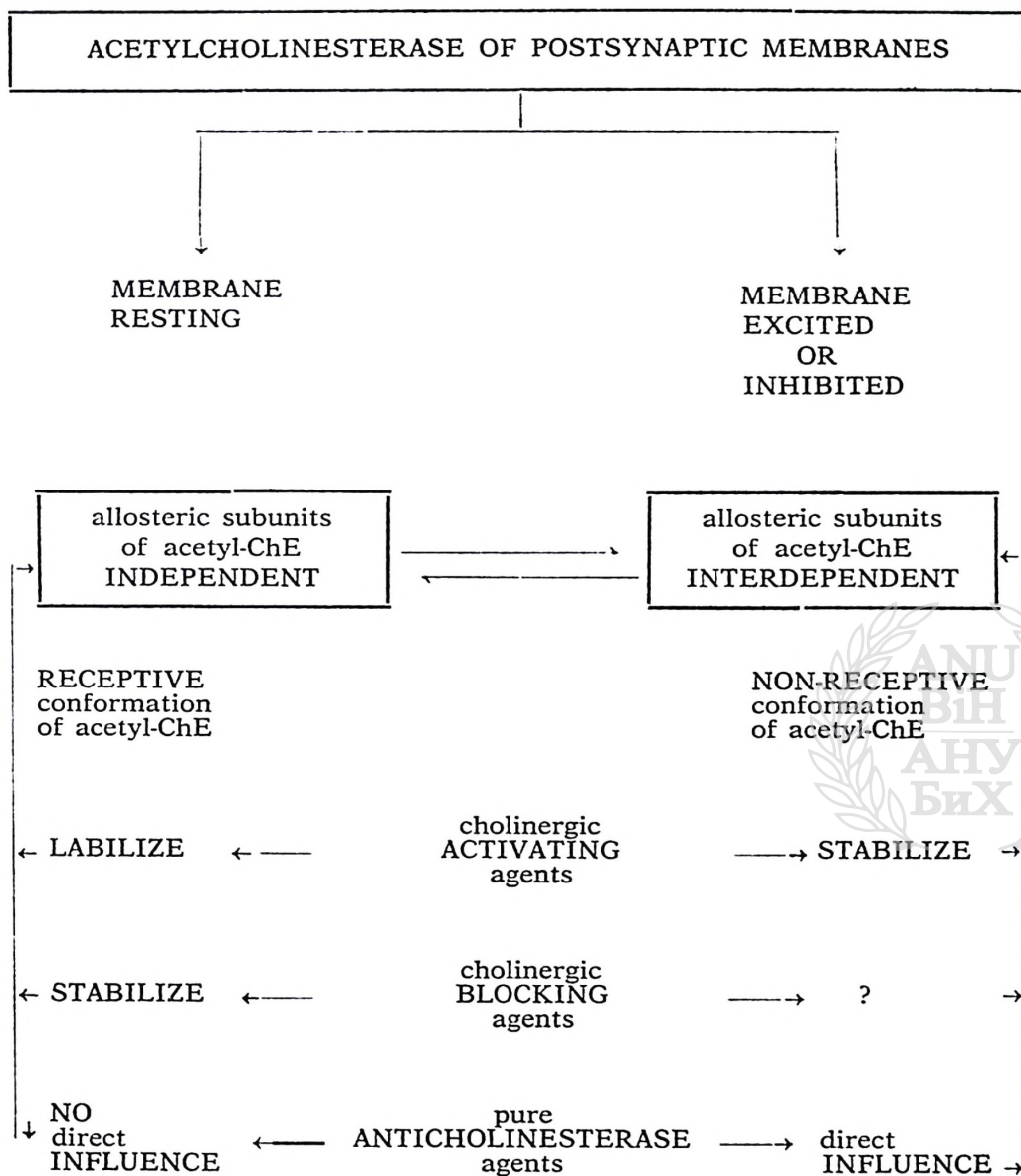
Thus, eleven tested substances influence the kinetics of acetyl-ChE of motor endplates in the same sense as they influence the kinetics of nicotine ChR: cholinergic activators induce a transition from the conformation with coöperatively dependent subunits ($n_H \sim 2$) to the conformation with independent subunits ($n_H \sim 1$); cholinergic blocking agents prevent such a conformational transition ($n_H \sim 2$), while substances with a pure anticholinesterase activity are indifferent to it ($n_H \sim 1$). The ability of cholinergic blocking agent to prevent the conformational transition of the membrane-bound acetyl-ChE protein, is independent of its inhibitor potency for this enzyme. Irrespective of their affinity for the esteratic site of acetyl-ChE, all tested cholinergic activating and blocking agents are bound with their cationic heads also to the anionic sites of this enzyme; we suggest that it is through these sites that cholinergic activating or blocking agents induce or prevent the conformational transition of the receptive enzyme.

Before turning to acetyl-ChE of the frog heart ventricle, it is worth noting that Atr, even in a 10 μ M concentration did not affect the kinetics of motor endplate acetyl-ChE. From Table 1 one can see that in the frog ventricle, too, acetyl-ChE with Ach as substrate obeys the Michaelis-Menten kinetics ($n_H \sim 1$). Since the heart ventricle responds to Ach with the muscarinic effect, Atr was chosen as cholinergic blocking agent; at a 0.1 μ M concentration, Atr altered the kinetics in the sense that n_H was lowered to ~ 0.5 .

In our interpretation, this means that the frog ventricle acetyl-ChE protein, before being influenced by Ach, exists in a conformation with interdependent subunits; this interdependence is, however, negatively coöperative, i. e., the binding of the ligand to one subunit lowers the affinity of the neighbour subunits for the ligand. Hence in our model, Atr blocks the effect of Ach on the heart ventricle because it stabilizes the negatively coöperative conformation of acetyl-ChE. So far, it is impossible to decide whether a negatively coöperative allosteric conformation is peculiar to either the muscarinic ChR or inhibitor ChR, or frog heart ventricle ChR. Methodologically, however, it is possible to clear up these alternatives.

The following tentative model of ChR function of acetyl-ChE is suggested (see Scheme 1):

Scheme 1



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**MODEL HOLINORECEPTORA OSNOVAN NA
KONFORMACIJSKIM PROMJENAMA ACETILHOLINESTERAZNE
BJELANČEVINE VEZANE ZA MEMBRANE**

KRATAK SADRŽAJ

Kinetičko ponašanje acetilholinesteraza integriranih u postsinaptičke membrane efektoru sa nikotinskim ili muskarinskim odgovorom na ACh studirali smo pod uticajem dvanaest supstancija; rezultate smo interpretirali u smislu konformacijskih promjena tog enzima: holinometrici su inducirali promjenu konformacijskog oblika sa međusobno zavisnim enzimskim podjedinicama u oblik sa nezavisnim podjedinicama; holinolitici su sprečavali ovaj prelaz, dok čiste antiholinesterazne tvari nisu uticale na njega. Zaključujemo da su acetilholinesteraze holinergičkih postsinaptičkih membrana alosteričke bjelančevine s osnovnim svojstvima hipotetičkih nikotinskih, odnosno muskarinskih holinoreceptora.

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RUDI PAVLIN

THE ACTIVITY OF CHOLINESTERASES AND MONOAMINE OXIDASE IN SINGLE NERVE CELLS AND GROUPS OF GLIAL CELLS ISOLATED FROM BASAL GANGLIA

The study of cholinesterase (ChE) and monoamine oxidase (MAO) activity in nerve and glial cells of basal ganglia might contribute to the present knowledge on basal ganglia functions and diseases. The activity of the two enzymes was already measured in patients suffering from Parkinson's disease and some authors found no difference from normal values (1,8). On the other hand, Weber (10) reported that in postencephalitic parkinsonism the values of ChE activity in basal ganglia were extremely low. In all these experiments, the enzyme activity was measured in homogenates of single nuclei or even of larger regions of the brain so that their results must represent the mean enzyme activity of all constituents of tissue homogenates.

The microdiver technique offers a possible approach to the study of enzyme activity in a single nerve cell or group of glial cells. The aim of our work is to find out whether there is a difference in ChE or MAO activity of single nerve cells and groups of glial cells isolated from different basal ganglia of 1. normal brain and 2. pathological brain (experimental parkinsonism or Parkinson's disease). This report deals with the first part of the program.

The experiments were done with healthy adult rats. The nerve cells and groups of glial cells were isolated according to Hydén and Pigon (5) and their ChE and MAO activity measured with the microdiver technique (2, 7, 11).

Figure 1

THE MEAN HYDROLYSIS RATE OF 3.0 mM ACh PER SINGLE NERVE
CELL OR GLIAL CLUMP OF THE RAT; $\mu\text{MOLES} \times 10^{-5} \pm \text{S. E. M.}$
SAMPLE/HOUR; $n = > 10$

Region	Cell	Glia
Caudate nucleus	1.4 ± 0.11	1.6 ± 0.04
Putamen	1.6 ± 0.11	1.3 ± 0.10
Globus pallidus	2.5 ± 0.25	1.3 ± 0.94
Substantia nigra	2.1 ± 0.11	1.4 ± 0.12
Reticular formation	2.0 ± 0.06	1.6 ± 0.08

Figure 1 shows the hydrolysis of acetylcholine (ACh) in single nerve cells and glial clumps isolated from a single basal ganglion. The enzyme activity seems to be rather uniform in all nuclei tested in both nerve cells and glia, whereas in the nerve cells of globus pallidus and substantia nigra a higher hydrolysis rate was observed. A comparison of the ChE activity of basal ganglion nerve cells with that of the reticular formation of the brain stem reveals practically no difference. The high rate of hydrolysis of ACh in glial tissue is striking.

Figure 2

THE MEAN HYDROLYSIS RATE — μMOLES
OF SUBSTRATE $\times 10^{-5} \pm \text{S. E. M. SAMPLE/HOUR}$

	Substrate (mM)		Caudate nucleus	Putamen	Globus pallidus	Substantia nigra	Reticular formation
Nerve cells	ACh 3.0		1.43	1.56	2.50	2.05	1.96
	BuCh 10.0		0.71	0.80	1.92	0.76	0.67
	MCh 3.0		0.16	0.89	0.89	1.38	1.38
Glial clumps	ACh 3.0		1.61	1.34	1.29	1.38	1.61
	BuCh 10.0		0.31	0.40	1.34	0.98	0.31
	MCh 3.0		0.80	0.67	1.29	1.20	0.13

Figure 2 shows hydrolysis rates of some choline esters. The glia of globus pallidus and substantia nigra as well as nerve cells from globus pallidus hydrolyze butyrylcholine at a higher rate than do the nerve cells and glia from other nuclei.

When compared with other nuclei globus pallidus is an exception also in respect to tyramine (Ty) oxidation (figure 3). Its nerve cells

Figure 3

THE OXIDATION RATE OF 10.0 mM Ty PER SINGLE NERVE CELL
OR GLIAL CLUMP OF THE RAT; $\mu\text{MOLES} \times 10^{-5} \pm \text{S. E. M.}$
SAMPLE/HOUR; $n = 10$

Region	Cell	Glia
Caudate nucleus	4.5 ± 0.53	1.5 ± 0.26
Globus pallidus	2.8 ± 0.20	1.4 ± 0.15
Substantia nigra	4.1 ± 0.52	1.5 ± 0.13
Reticular formation	4.8 ± 0.23	1.6 ± 0.16

oxidize Ty at lower rate than do the cells isolated from other basal or reticular nuclei where a relatively uniform rate was found. The oxidation rate of Ty in cells is 2—3 times higher than the hydrolysis rate of ACh. The oxidation of Ty by glial tissue is very slow but uniform in all tested nuclei.

Measuring the Ty oxidation in cells and glia isolated from one nucleus, one obtains a picture similar to that obtained with ACh hydro-

lysis (7): a wide variation in enzyme activity (figure 4). In glial samples the MAO activity is sometimes absent or below the sensitivity of the method.

Figure 4

NERVE CELLS AND GLIAL CLUMPS ISOLATED FROM THE CAUDATE NUCLEUS OF THE RAT. 10.0 mM Ty; $\mu\text{MOLES} \times 10^{-5}$ SAMPLE/HOUR

Cell	Glia
4.7	0.7
4.2	0.6
5.5	2.2
3.4	1.7
5.4	2.3
2.1	0
2.7	1.6
4.4	2.2
3.0	3.5
2.7	0.7
4.8	0.4
4.1	0.5
4.3	0
2.6	1.6
1.8	0
Mean $\pm$ S. E. M.:	4.5 $\pm$ 0.53
	1.5 $\pm$ 0.26

Figure 5

ONE AND THE SAME RETICULAR PONTINE NERVE CELL,
TWO SUBSTRATES IN SUCCESSION: 3.0 mM ACh AND 10.0mM Ty.
THE ENZYME ACTIVITY IN $\mu\text{MOLES} \times 10^{-5}$ CELL/HOUR

Cell. No.	First determination: hydrolysis of ACh	Second determination: oxidation of Ty
1	3.4	2.1
2	4.2	5.7
3	2.2	8.9
4	3.4	3.9
5	1.7	1.7
6	2.4	3.0
7	2.9	2.4
Mean $\pm$ S. E. M.:	2.9 $\pm$ 0.58	4.0 $\pm$ 0.70

Cell No.	First determination: oxidation of Ty	Second determination: hydrolysis of ACh
1	7.2	3.6
2	6.8	4.9
3	5.9	1.6
4	4.2	5.8
5	3.6	2.6
6	3.8	3.6
7	4.2	2.5
Mean $\pm$ S. E. M.:	5.1 $\pm$ 0.44	3.5 $\pm$ 0.56

Those experiments in which the hydrolysis of ACh and the oxidation of Ty were measured in succession in the same nerve cell revealed that every nerve cell studied had the ability of inactivating either substrate (figure 5).

It has long been known that the basal ganglia constitute the brain region with the highest ChE activity (6, 3). It is possible that the considerable capacity of glial tissue of basal ganglia to hydrolyze choline esters (as seen in figure 2) substantially contributes to the high ChE activity obtained with the homogenates of this region.

The somewhat peculiar behaviour of ChE and MAO in globus pallidus as compared with that of the other basal ganglia can not be interpreted in the light of the existing information on function of this nucleus.

Consolo, Giacobini and Karjalainen (4) measured the oxidative deamination of Ty in single ganglion cells of the cat using a radioactive method and found 5.5×10^{-12} moles of deaminated Ty per cell per hour. This result is about 10 times lower than that yielded by our experiments; the fact however must not be lost sight of that they used a different technique, another species and another brain region. The above authors observed a wide variation in the results, a variation ranging from 1 to 40×10^{-12} moles of substrate (cell) hr. The former observation was made also in our experiments (figure 3).

The finding in the same nerve cell of two active enzymes which are involved in neurotransmission might be related to the observation of Salmoiraghi and Stefanis (9) who in microelectrophoretic experiments evoked a response in the same nerve cell with the injection of ACh or noradrenaline.

To summarize, the hydrolysis rate of ACh and the oxidation rate of Ty in single nerve cells and groups of glial cells isolated from basal ganglia of the rat were measured. The rate of activity of these enzymes in cells of different basal nuclei was found to be rather uniform. An exception was the globus pallidus where a higher rate of ACh hydrolysis and a lower one of Ty oxidation was obtained. Every investigated cell had the ability to hydrolyze ACh and oxidize Ty.

Acknowledgements

The author thanks Mrs. Ida Balaž for her skillful technical assistance. This investigation was supported by the Boris Kidrič Foundation, Ljubljana.

RUDI PAVLIN

AKTIVNOST HOLINESTERAZA I MONOAMINOOKSIDAZE U POJEDINIM NERNVIM ČELIJAMA I GLIOZNIM GRUDVICAMA IZOLOVANIM IZ BAZALNIH GANGLIJA

KRATAK SADRŽAJ

Poznavanje aktivnosti holinesteraza i monoaminooksidaze u pojedinim nervnim ćelijama izolovanim iz bazalnih ganglija doprinelo bi našem poznavanju mehanizama bolesti bazalnih ganglija. U pojedinim

nervnim ćelijama i glioznim grudvicama iz bazalnih ganglija mjerili smo hidrolizu nekih holinskih estera i oksidaciju tiramina. Veći deo merenja obavili smo na zdravim mozgovima štakora, a neke i na zdravom čovečjem mozgu. Upotrebljavali smo mikromanometričnu kartezijsku tehniku. Našli smo da je brzina hidrolize acetilholina u gliji bila skoro jednaka onoj u nervnim ćelijama, međutim oksidacija tiramina u gliji dostigla je otprilike jednu trećinu brzine u nervnim ćelijama. Nervne ćelije i glija iz globusa pallidusa i supstance nigre hidrolizirale su butirilholin brže od nervnih ćelija i glije izolovanih iz drugih jezgara. Eksperimenti u kojima smo merili hidrolizu acetilholina i oksidaciju tiramina naporedo u istoj nervnoj ćeliji pokazali su da je svaka nervna ćelija imala enzimске aktivnosti za hidrolizu acetilholina i za oksidaciju tiramina.

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DISCUSSION

Bruggencate: Dr Pavlin, could you give me the dimension of the orifice of the pipette with which aspirated you the cells? Furthermore, it is difficult to discern neurones from glial cells?

Pavlin: The dimension of the glass pipette is about 50 microns. To the second question: staining the tissue with methylene blue allows taking an easy difference between these two types of cells.



**Clinical studies
in human parkinsonism**



M. R. DIMITRIJEVIĆ, F. GRAČANIN, I. MARINČEK,
T. PREVEC, J. K. TRONTELJ AND J. V. TRONTELJ

A STUDY OF THE EFFECTS OF CONTROLLED AFFERENT INFLOW ON TREMOR IN PARKINSONIAN PATIENTS

Several studies on Parkinsonism, to mention only a recent one by Hoehn and Yahr (2), have shown that tremor is the most frequent initial symptom. There is no wonder, therefore, that so many both clinical and research workers interested in Parkinsonism focused their attention on tremor.

In our study we were concerned with so-called static tremor appearing in the relaxed patient as the well-known pill-rolling forearm and finger movements.

EXTENSORS OF THE WRIST

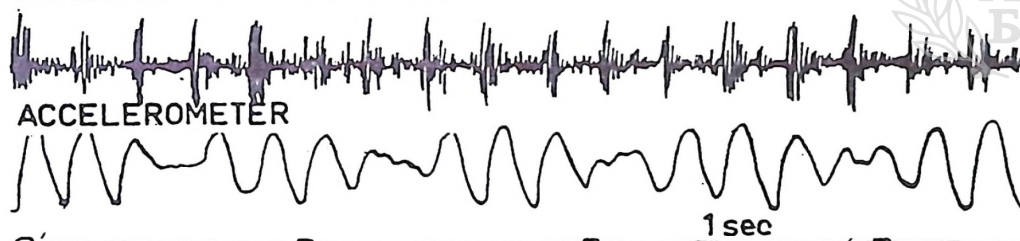


Fig. 1

Electromyographic and accelerometric recording of tremor.

Figure 1 is an EMG recording of the tremor, showing its rather regular rhythmicity.

Our study had two goals: The first one was modest as regards scientific achievements and was just to develop a method of recording and computation of EMG and accelerometric parameters of the tremor, accurate enough to achieve the next goal; and this was to see whether this parameter can be influenced by adding some sensory information incorporated into a feedback loop.

MATERIAL AND METHOD

The study included 5 patients aged 50 to 60, suffering from uncomplicated Parkinsonism for 4 years or longer. We have satisfactory recordings from three of these.

The EMG was detected by surface electrodes over the flexors and extensors of the wrist and fingers. Two accelerometers were placed in perpendicular directions on the back of the hand.

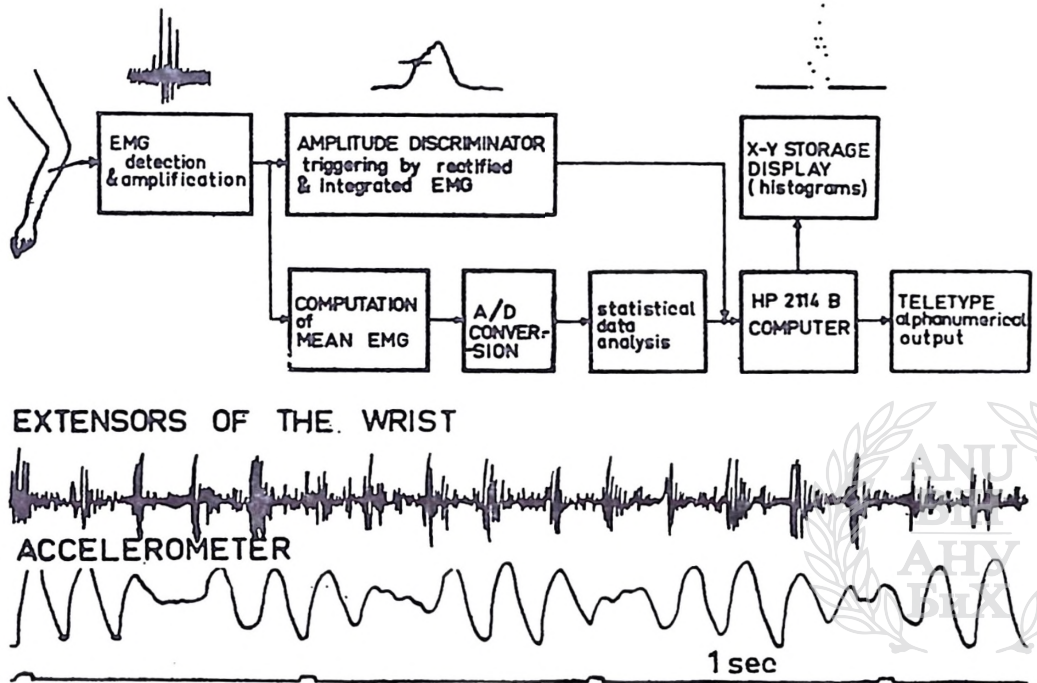


Fig. 2

Block diagram of the experiment.

The EMG and the accelerometers were automatically and on-line processed in the way symbolically shown in figure 2. Several of the methodological and technical solutions used here are original innovations by our engineer, Mr. Janez Trontelj.

RESULTS

Table 1 shows the results of such an analysis. From mean EMG you can see that relaxation combined with mental effort gave the strongest tremor, while voluntary movement depressed it. In fact, nothing very new is in this table; it is shown just as an example of how our analysis works.

Table 1.

MEAN FREQUENCY OF TREMOR AND MEAN EMG OF BURSTS
IN TWO PARKINSONIAN PATIENTS

PATIENT	EXPERIMENTAL SITUATION	NUMBER OF BURST	MEAN FREQUENCY OF TREMOR	MEAN EMG OF BURSTS	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
TRP 2	RELAXATION HAND PRONATED	250	4.6	336	99	0.29
	RELAXATION HAND SUPINATED	250	5.1	141	59	0.4
	ANTE-FLEXION HAND PRONATED	250	4.3	229	24	0.1
	CALCULATION HAND PRONATED	250	4.9	434	113	0.26
	RELAXATION HAND PRONATED	250	4.9	297	76	0.25
	CALCULATION HAND PRONATED	250	4.7	426	90	0.2

Now we wanted to see whether or not it is possible to change the parameters of the tremor by introducing a purely afferent inflow.

We chose a 20 ms lasting train of high frequency electrical pulses applied to the median or radial nerve. Such a stimulus gave a kind of a paraesthetic sensation, but was in all cases too weak to give any direct or indirect motor effect.

The stimulus was triggered by the tremor itself, the triggering signal being generated when the acceleration or the rectified and integrated EMG burst reached certain level.

The actual stimulus was delayed after the triggering signal by 35, 70 or 105 miliseconds. In this way our artificial sensory inflow was in a feedback loop with the oscillating activity of motoneurons (fig. 3).

What happened to tremor as a result of such feedback stimulation was a change in tremor frequency and mean tremor EMG (figs. 4 and 5): the frequency of the tremor was significantly increased, while mean EMG of its bursts diminished. The important fact here is that only one of the three delays was really effective: 35 milliseconds after the triggering point on the rising slope of the acceleration signal. The effect was very constant and was proportional to the strenght of the stimulus. With the other two delays, even 50% increased stimulus showed little effect.

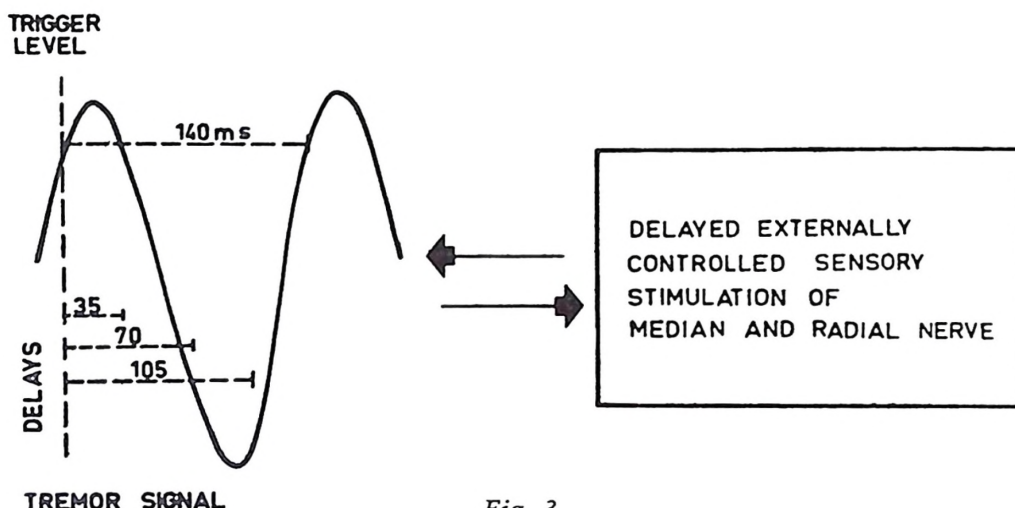


Fig. 3

Generation of trigger signal by tremor acceleration.

RELAXATION

MEAN = 4.7 ± 0.2 Hz

30 40 50 60 Hz

SENSORY STIMULATION

MEAN = 6.5 ± 0.2 Hz

50 60 70 80 Hz

INSTANT RATE OF TREMOR

Fig. 4

Change in frequency of the tremor due to sensory stimulation of the median nerve (train: 1000 Hz, 20 ms, 75 Volts; delay after trigger signal 35 ms).

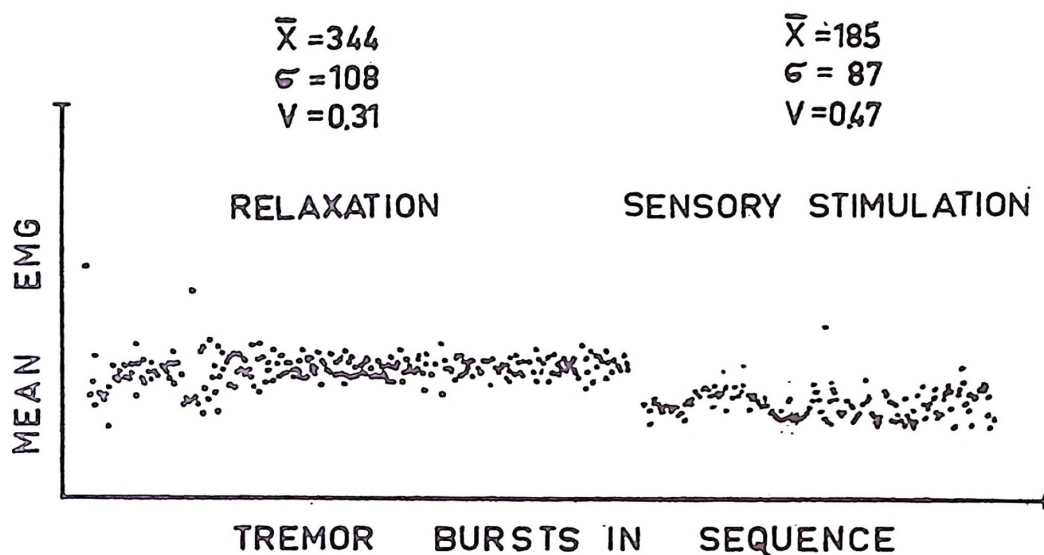


Fig. 5

Change in mean EMG of tremor bursts during sensory stimulation of the median nerve (train: 1000 Hz, 20 ms, 75 Volts; delay after trigger signal 35 ms).

DISCUSSION

If any preliminary conclusion can be drawn from this very preliminary work, then this is the suggestion that the Parkinsonian tremor can be modified by certain sensory inflow, appropriately phase-locked to the tremor. The emphasis is on appropriately, and it does seem that the interval during which the system is sensitive is rather rigidly determined.

Discussing this phenomenon I have to mention another observation made by our group a few years ago: we found that sensory stimulation can suppress ankle clonus in hemiplegic patients (1). Here again, the effective parameters of stimulation were highly specific. This interesting analogy, of course, can not be taken to point to a common pacemaker for both tremor and clonus, but it does prove some degree of dependence of either phenomenon on the afferent inflow.

M. DIMITRIJEVIĆ, F. GRAČANIN, MARINČEK,
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PROUČAVANJE UČINKA KONTROLISANOG AFERENTNOG UTICAJA NA TREMOR PACIJENATA OBOLJELIH OD PARKINSONOVE BOLESTI

KRATAK SADRŽAJ

U ovoj studiji pokušalo se ustanoviti, dali je moguće promjeniti parametre parkinsonskog tremora sa uvođenjem senzorne informacije uklopljene u povratnu vezu.

Elektromiografski i akcelerometrički parametri tremora bili su automatsko analizirani sa kompjuterom; u različitim fazama akcelerometričkog signala odnosno perioda elektromiografske aktivnosti na nervus medianus ili radialis apliciran je električki nadražaj ispod praga za direktni ili indirektni motorni odgovor. Pokazalo se, da je na ovaj način moguće značajno uvećati frekvenciju i smanjiti veličinu periodičke EMG aktivnosti, ali samo kad je nadražaj apliciran u tačno određenoj fazi tremora. Ukazano je na analogiju sa klonusom kod paraplegičara, na koji se slično može utjecati samo sa senzornim stimulusom specifičnih vremenskih parametara.

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M. R. DIMITRIJEVIĆ, J. FAGANEL AND M. JANKO

OBSERVATIONS ON VARIABILITY OF TENDON REFLEXES IN PARKINSONIAN PATIENTS

The tendon jerks have been used in human neurophysiology as a tool in the studies of a variety of abnormal conditions for over 70 years. Their use has particularly expanded in the last decade, which has brought advanced technical facilities for their accurate eliciting, recording and analysis, and significant improvements in the background physiological knowledge.

More than 15 years ago, Struppler and Fleischhauer published a study on tendon jerks in patients with hemiparkinsonism (5). They were able to demonstrate a decrease in tendon jerks on the affected side, while there was no difference between the two sides in the H-reflex, which is the electrically induced counterpart of the tendon jerk, by-passing the gamma loop.

This interesting observation was later confirmed by Meltzer, Hunt and Landau (3). Arthur Ward took these findings as experimental support to his model of Parkinsonian syndrome based on partial interruption of gamma excitatory circuits (6).

However, more recently there appeared an elaborate microneurographic study by Hagbarth and associates who demonstrated exaggerated discharging of Ia fibres in Parkinsonian patients, hence hyper- rather than hypoactivity of the gamma system (2).

It is the teaching of clinical neurology that the tendon jerks in Parkinsonism tend to be normal, though rigidity may render them difficult to elicit and reduced in amplitude. It is also said that in the early stages they may actually be increased (1, 4).

The premise of our own study on which we wish to report here was that, for the stretch reflex to be elicited, one has to have an active gamma loop biased to spindle primaries, although the experimentally proved alpha-to-gamma linkage might well compensate for the partial interruption of the gamma system.

On the other hand, the size of the tendon jerk should depend on the spinal interneuronal system, which is known to influence motoneurone excitability, in addition to the supraspinal descending excitatory and inhibitory pathways. Our previous studies on normals and hemiple-

gic and paraplegic patients have shown that some insight into the function of these structures can be obtained by testing the fluctuation of spinal motoneurone excitability by repetitively elicited tendon jerks.

Indeed the comparison of these conditions with Parkinsonism revealed interesting differences.

MATERIAL AND METHOD

The results to be presented were obtained in five patients suffering from Parkinsonism for 4 years or longer. Their ages are between 50 and 60 years and none of them had any clinical or neurophysiological evidence of other neurological disorders. In all of them hypokinesia and rigidity were the most prominent symptoms.

The taps to the patellar tendon were delivered by a specially designed electrodynamic hammer with controllable force and repetition rate. An in-built pressure sensitive element reported the effective force of the head for each blow.

The EMG reflex responses were picked up by surface electrodes from the quadriceps muscle, amplified, rectified and integrated with an originally constructed hybrid unit and fed into an HP 2114 B computer. The output of the pressure-sensitive element of the hammer was subjected to the same procedure. Means, standard deviations and histograms, as well as cross-correlation between the two functions were computed on-line (the technical part of the experimental design and the original constructions have been accomplished by our engineer Mr. Janez Trontelj).

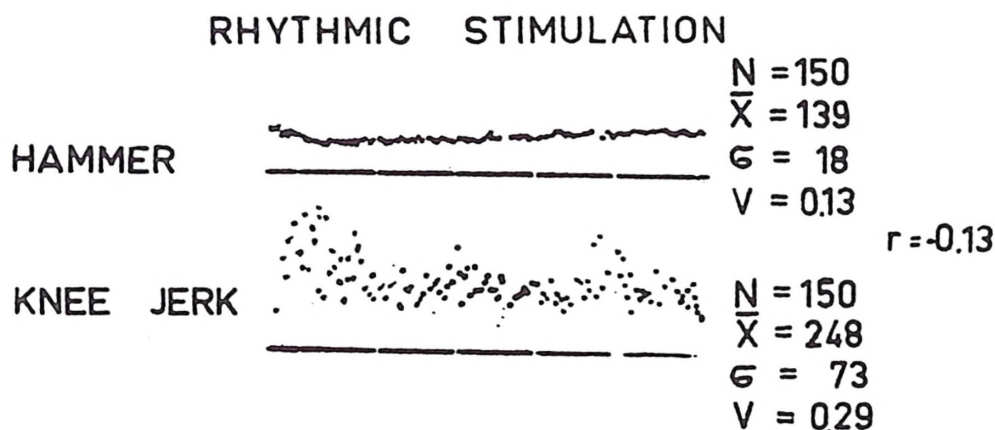


Fig. 1

Repetitively induced knee jerks in a normal subject. Upper trace: hammer force; lower trace: mean EMG of individual jerks.

RESULTS

Figure 1 shows the results of this procedure in a normal person. One hundred successive jerks elicited at a regular frequency of 1 per second show typical degree of scattering of the size. As you can see this is considerable, although all controllable experimental conditions were kept as constant as possible. Particular attention was paid to achieve as complete relaxation of the subjects as possible and a uniform level of wakefulness. (From our previous studies we were well aware of extensive changes in the size of the tendon jerks during drowsiness and sleep) (fig. 2).

Before presenting the results in the Parkinsonian patients, it will be of interest to see what happens in complete paraplegia (fig. 3) and in hemiplegia — the patient had a stroke 2 years before (fig. 4).

In sharp contrast to these conditions is what we found in the Parkinsonian patients (fig. 5). As you can notice, the variation of the size of repetitive jerks here is much wider. That this was not due to the inconstancy of the taps is shown on figure 6 and there was no correlation between the strength of the tap and the size of the jerk.

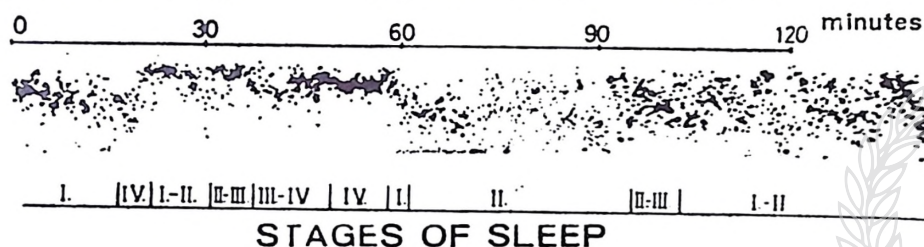


Fig. 2

Repetitively induced (0,2 Hz) Achilles jerks in a normal subject during different stages of sleep.

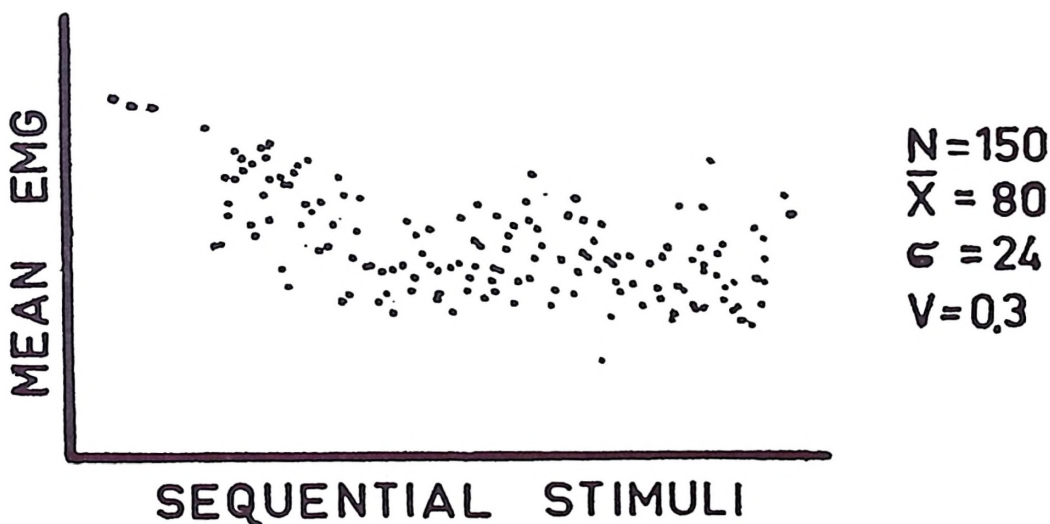


Fig. 3

Repetitively induced knee jerks in a patient with complete paraplegia.

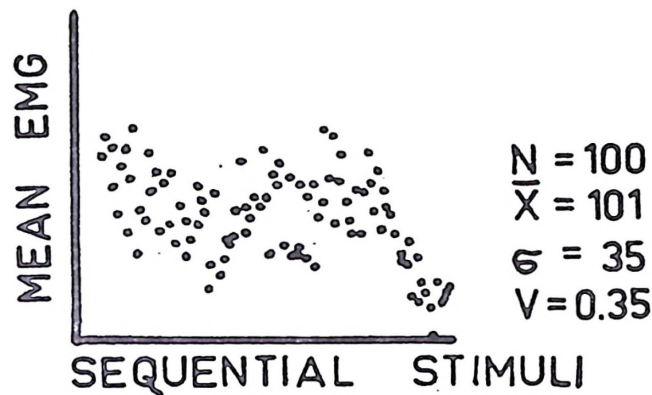


Fig. 4
Repetitively induced knee jerks (the affected side) in a hemiplegic patient.

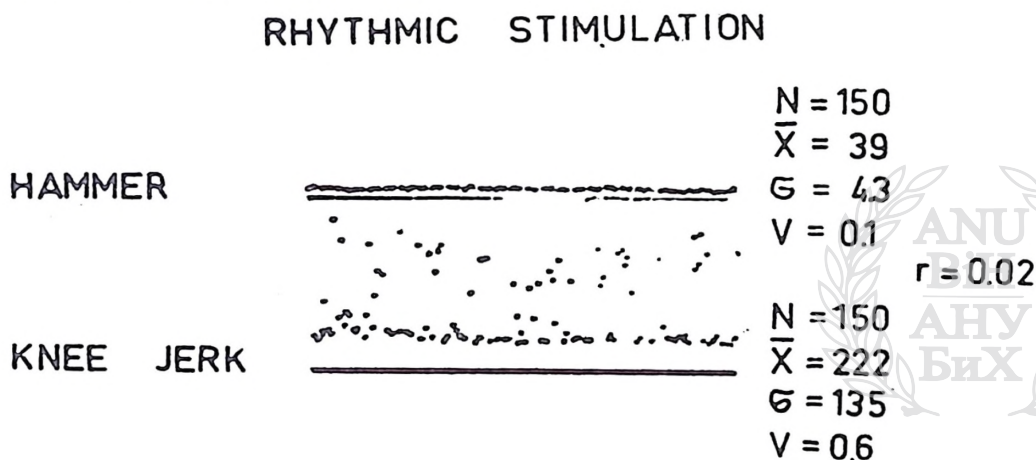


Fig. 5
Repetitively induced knee jerks in a Parkinsonian patient. Upper trace: hammer force; lower trace: mean EMG of individual jerks.

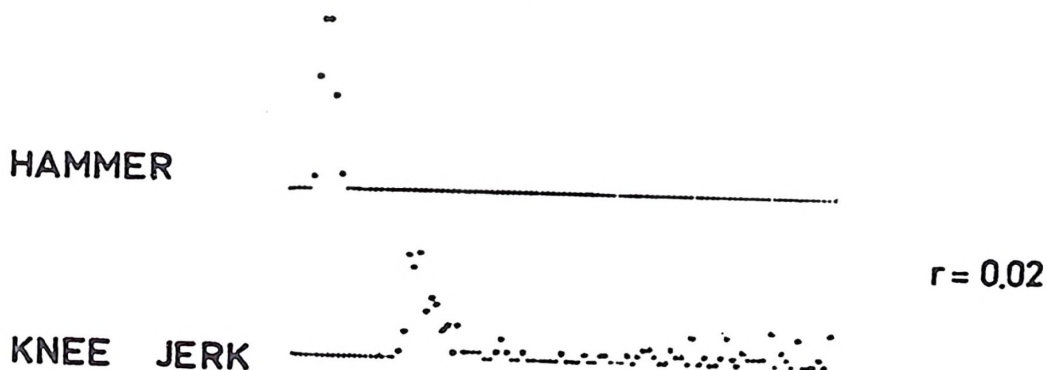


Fig. 6
Distribution of hammer forces (upper trace) and EMG responses (lower trace). r = coefficient of correlation.

There was striking consistency of the results in all of the 5 patients, as you can see from table 1.

Table 1.

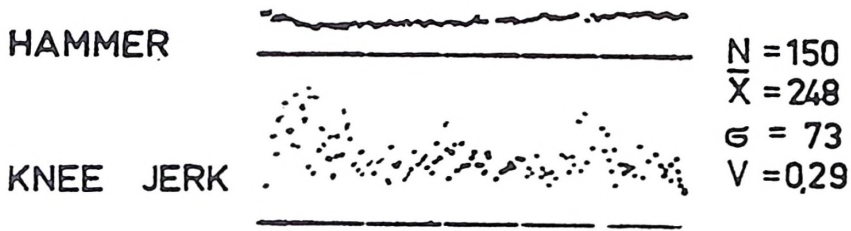
MEAN EMG RESPONSE TO RHYTHMIC STIMULATION

PATIENT	NUMBER OF STIMULI	MEAN EMG RESPONSE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
SPP 1	100	363	85	0.23
	100	342	88	0.26
	150	273	100	0.36
SPP 4	100	262	76	0.29
	250	217	70	0.32
	150	291	105	0.36
SPP 5	100	325	109	0.33
	250	272	99	0.36
	150	190	40	0.21
SPP 6	100	176	22	0.12
	250	160	94	0.58
	150	222	135	0.60
SPP 7	100	214	123	0.57
	250	239	133	0.55
	150	222	135	0.60

It must be admitted that this type of findings is somewhat surprising: it indicates a very rich fluctuation of the excitability of spinal motoneurons, which would seem to stand in a sharp contrast to the cardinal clinical symptoms of these patients: poverty and slowness of voluntary movement, and rigidity.

In an attempt to elucidate this contradiction, we made another type of experiments in which the intervals between the successive tendon taps were randomly changed. The range of these changes was between 0.25 and 2 seconds. This procedure greatly increased variation of the size of the jerks in normals, hemiplegics and paraplegics. Figure 7 shows what happens in a normal subject; according to our interpretation, the increased variation in this case is primarily due to the changes induced by random stimulation in the spinal interneuronal system.

STIMULATION RHYTHMIC



STIMULATION RANDOM

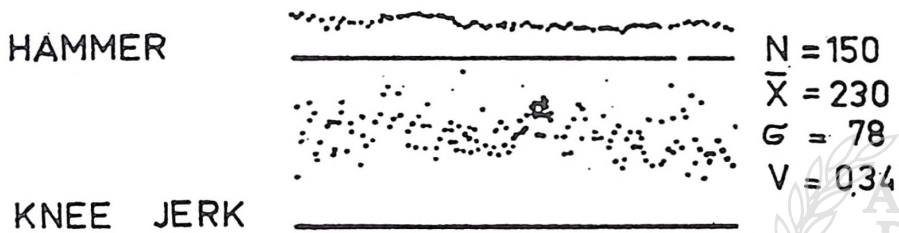


Fig. 7

Responses to tapping at regular and random intervals in a normal subject.

Table 2.

MEAN EMG RESPONSE TO STIMULATION AT RANDOM INTERVALS

PATIENT	NUMBER OF STIMULI	MEAN EMG RESPONSE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
SPP 1	100	188	81	0.43
	100	345	79	0.23
SPP 4	250	323	98	0.30
	250	278	109	0.39
SPP 5	250	354	95	0.26
	250	354	96	0.27
SPP 6	250	189	93	0.49
	250	122	24	0.19
SPP 7	250	236	123	0.55
	250	258	135	0.52

Again, the Parkinsonian patients behaved quite differently (fig. 8). Table 2 shows no obvious differences between the effects of rhythmical and random stimulation.

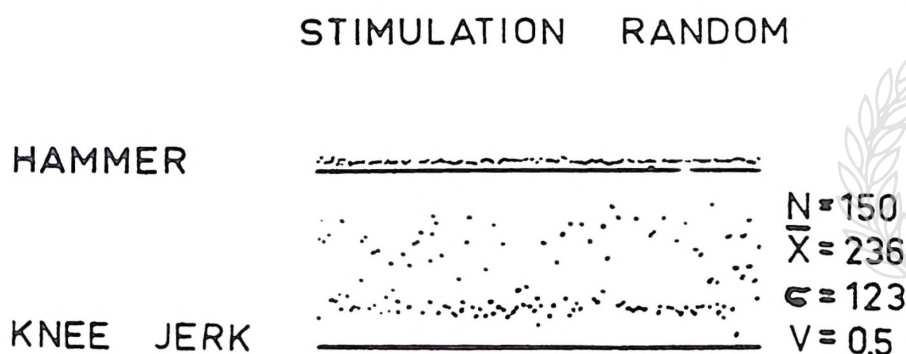
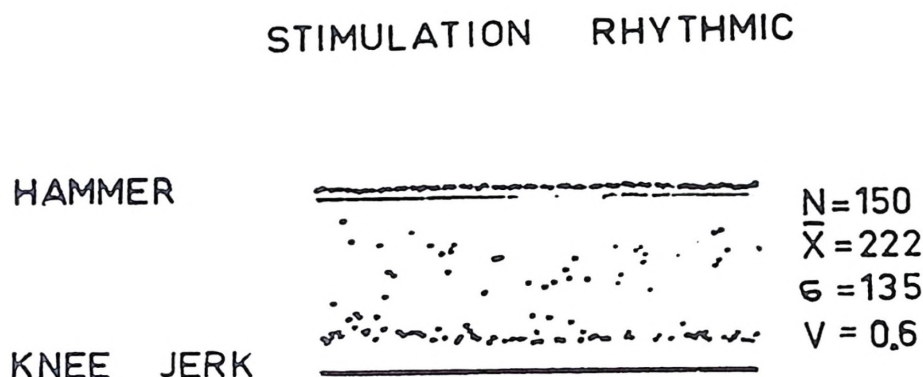


Fig. 8

Responses to tapping at regular and random intervals in a Parkinsonian patient.

CONCLUSIONS

Presented results indicate a very rich fluctuation of spinal motoneurone excitability in the Parkinsonian patients studied; this is in sharp contrast to the clinically observed hypokinesia and rigidity; and it is little influenced by the changes in the afferent information code. It seems as if the spinal motor cells and their primary and interneurone afferents were subordinated to abnormally strong supraspinal control, which however does not preclude rich fluctuation of spinal motoneurone excitability, the latter again being of some supraspinal origin.

O VARIJABILITETU TETIVNOG REFLEKSA KOD PACIJENATA SA PARKINSONOVOM BOLEŠĆU

KRATAK SADRŽAJ

Kod ritmičnog izazivanja tetivnog refleksa veličina elektromiografskih odgovora pokazuje varijacije koje među ostalim odražavaju promjene u ekscitabilnosti donjeg motornog neurona. U ovom radu uspoređivan je varijabilitet tetivnog refleksa kod normalnog čovjeka, paraplegičara, hemiplegičara i pacijenata sa Parkinsonovom bolešću. Kod posljednjih su nađene neočekivano bogate fluktuacije ekscitabilnosti donjeg motornog neurona, što je u oštrom kontrastu sa kliničkom hipokinezijom i rigidnošću. Promjene u kodi aferentne informacije sa slučajno promjenljivim intervalima između stimulusa kod parkinsoničara nisu imale značajnog utjecaja. Izgleda kao da su spinalne motorne ćelije i njihovi primarni i interneuronski aferentni putevi podvgrnuti pretjerano jakoj supraspinalnoj kontroli, koja ipak ne sprečava bogate, vjerojatno supraspinalno determinirane fluktuacije ekscitabilnosti spinalnih motoneurona.

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DISCUSSION

Struppler: May I ask, what time intervals you used between the single mechanically evoked responses? As you know, repetitive responses are remarkably influenced by a long lasting depression you certainly were aware of.

Dimitrijević: We were aware of the phenomenon described as low frequency depression. For this reason we used two different patterns of stimulation: one with regular intervals between the stimuli and the other one with random intervals ranging between 0.25 and 2 sec. We have observed

that stimulation of this latter type prevents or delays the development of low frequency depression. We did observe some gradual decline in the size of responses on regular stimulation in normals (cf. Fig. 1), but we could not see any decline in the Parkinsonian patients even when the stimulation was regular.

Struppler: I would like to ask you, if you have recorded in your interesting investigations the amount of rigidity simultaneously with T-reflex. As you know the T-reflex response depends not only upon the excitability of the α -motoneurons, but also on the amount of stretch, which depends on the length of the muscle. Therefore I am wondering whether changes in stretch might contribute to the variation of the reflex responses.

Dimitrijević: The pressure-sensitive element built in the hammer head reported the pressure developed at the tendon by the individual blows; there was no correlation between the pressures and the mean EMG's. Therefore we believe that the state of the tendon did not contribute to the large fluctuation we observed in the Parkinsonian patients.



MILAN GREGORIC

NEUROPHYSIOLOGICAL ANALYSIS OF THE BLINK REFLEX IN PARKINSONISM

Electromyographic studies of blink reflex elicited by taps to periorbital region have shown that it consists of two components: of an early response which is rather constant, and of later response with highly variable parameters. Both components can also be observed on electrical stimulation of the supraorbital nerve. In normal subjects, rhythmic stimulation results in rapid habituation of the second component. On tapping the glabella, reflex blinking normally stops after first few taps. In contrast to this, some Parkinsonian patients continue to blink on every tap for an indefinite period of time. This sign has been used by some neurologists as an early indication of Parkinsonism. Rushworth on the basis of his EMG studies decided that this was due to the persistence of the second component, which did not habituate at all (10). In contrast to this opinion is the observation by Struppler and Dobbelsstein that the second component habituates in the usual way (13). These two authors thought that the persistence of blinking was due to an abnormal increase in the first component.

In our own studies, the starting points of which were the works by Rushworth, Struppler, and others, we tried to analyse the neurophysiological parameters and habituation of the blink reflex in patients with extrapyramidal syndromes and in healthy people. The blink reflex was repetitively elicited by mechanical and electrical stimuli to observe the changes developing with repetition.

MATERIALS AND METHODS

For mechanical stimulation we used an electrodynamic hammer delivering taps to the glabella at a constant strength and a repetition rate of one per second.

Electrical stimulation was performed through bipolar stimulating electrodes affixed to the skin over the right eye. The stimulus was a two milliseconds lasting train of pulses and was repeated at a rate of one per second as well.

The intensity of both stimuli was individually adjusted so that the minimal intensity to give a detectable motor response was increased by 50 per cent.

The EMG responses were detected by a pair of surface electrodes placed on the lower margin of the right orbit. After suitable amplification they were rectified and integrated by an originally constructed hybrid unit (14) connected to an averaging computer. The results were displayed and printed out in the form of sequential histograms of magnitudes of individual EMG responses.

An accelerometer was affixed to the skin over the upper part of the orbicularis oculi muscle to record mechanical movements.

The study included 10 normal subjects, 9 Parkinsonian patients, 2 patients with Huntington's chorea and one patient with dystonia musculorum deformans.

All the Parkinsonian patients exhibited marked immobility of facial muscles and a reduction in spontaneous blinking.

RESULTS

In the control group of normal subjects the blink reflex behaved in the manner described by Kugelberg, Rushworth and others. Repetitive stimulation had little effect on the first component and its temporal parameters, although the magnitude was somewhat variable. The longer lasting second component, however, underwent rapid habituation. In 8 subjects it disappeared altogether after the third tap. In only 2 subjects the initial rapid decrease was followed by occasional appearing till the end of the series. Visible blinking was correlated to the second component and disappeared together with its cessation, in spite of the persistence of the first component (fig. 1 and 2).

In contrast to the results in the normal subjects, in four out of our 9 Parkinsonian patients the first component was extremely increased in duration, while other parameters remained unchanged.

In 5 patients habituation of the second component was very slow or was not manifested at all.

It was only in two patients that the first component was of normal duration and the second component showed rapid habituation.

In other words, there were three types of behavior of the repetitively induced blink reflex in the Parkinsonian patients.

One was characterized by an extremely prolonged first component and normally habituating second component (Fig. 3 and 4).

The second type showed a moderately prolonged or normal first component and an incompletely habituating second component. In this case the blinking persisted as a result of both components, or predominantly of the second component (Fig. 5 and 6).

The third type with its short first component and rapidly habituating second component did not differ from the normal condition. Here the blinking disappeared together with the second component.

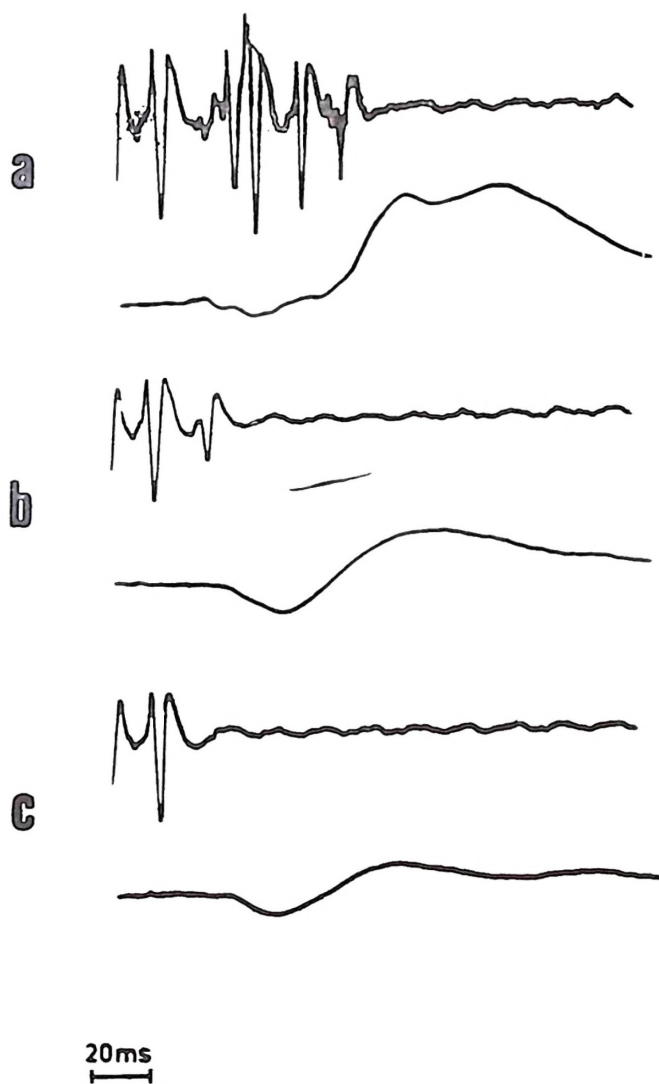


Fig. 1

The blink reflex of a normal person elicited by taps to the glabella. The upper traces are the EMG, and the lower traces are mechanical recordings by an accelerometer. The first three responses (a, b, and c) in a series are shown. Note the rapid habituation of the second component followed by virtually complete disappearance of blinking, even though the first component persists. Calibration in the lower traces is here 10 msec.





Fig. 2
A sequential histogram of a series of reflexes in a normal subject showing changes in the size of the first component (left) and rapid decrease of the second component (right).

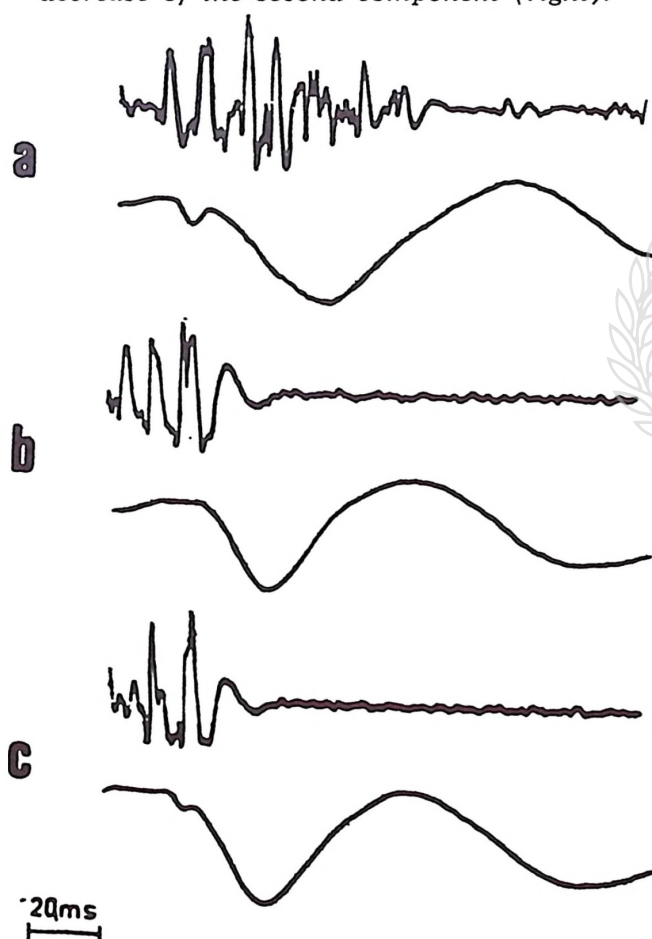


Fig. 3
The persistence of blinking due to an abnormally prolonged first component. The second component habituates normally. The first three responses of a series (a, b, and c).

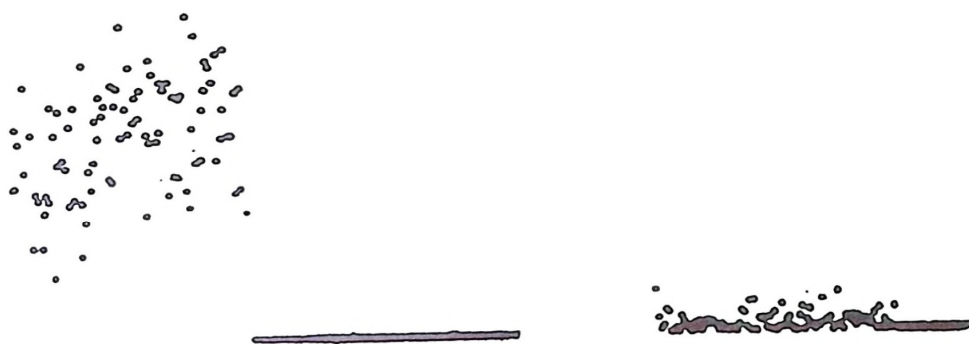


Fig. 4

A sequential histogram of EMG in a series of reflexes of the type shown in Fig. 3. The first component is shown on the left, and the second on the right side.

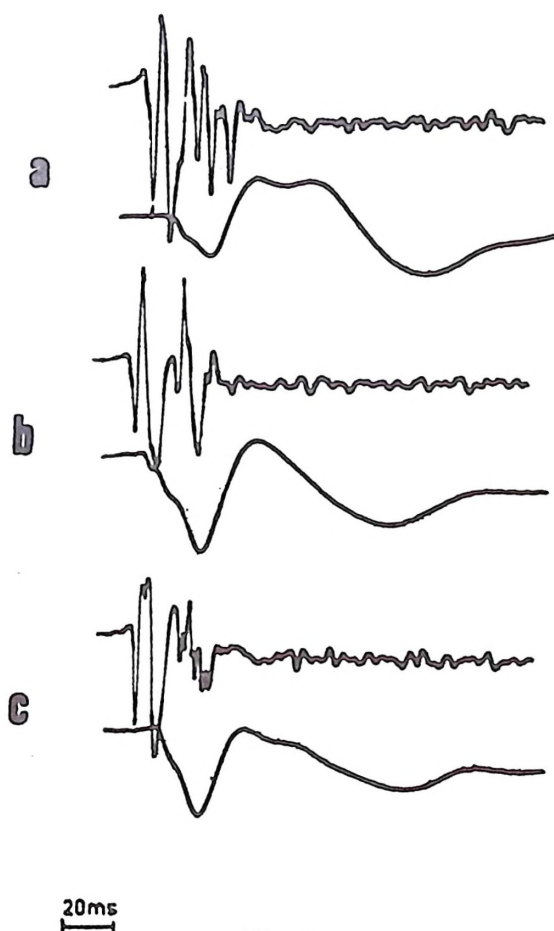


Fig. 5

The first three responses (a, b, and c) of a series of reflexes. The persistence of mechanical contractions is due to a moderate increase in the first component as well as to the slowly habituating second component.





Fig. 6

A sequential histogram of the first (left) and second (right) EMG components of a series of reflexes of the type shown in Fig. 5. The second component habituates incompletely.

In one of the two patients with Huntington's chorea the first component was slightly prolonged, but the second component habituated normally in both patients (Fig. 7). This is in contrast to the findings by Struppler who reported a decrease in both components, but particularly in the second (12).

In only one patient with Dystonia musculorum deformans no abnormalities could be observed either in the parameters of the first or in habituation of the second component.

DISCUSSION AND CONCLUSIONS

The nature of the first component of the blink reflex is still a matter of controversy. Most authors consider it a proprioceptive or myotatic reflex, but others object emphasizing the lack of physiological and anatomical evidence for this explanation. According to Shahani this component may represent a highly organized cutaneous reflex (12). At the present stage of knowledge, it is not easy to decide which of the two hypothesis may be correct or more correct. It would be even more difficult to explain why the first component becomes prolonged in some Parkinsonian patients. Also, it is not known, whether or not this prolongation may be regarded a specific feature of Parkinsonism.

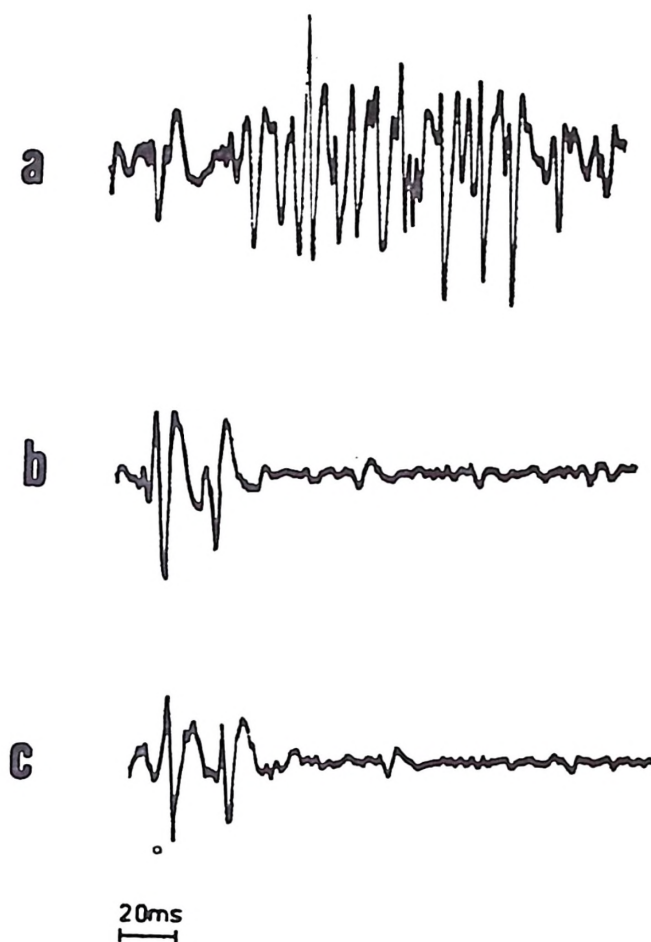


Fig. 7

The first three EMG responses of a series of blink reflexes in a patient with Huntington's chorea. The first component is slightly prolonged, while the second component habituates normally.

As to the second component, it is generally accepted to represent a kind of a defense reaction to the noxious stimulus. According to Larson and other authors it is strongly influenced by various psychic factors (8). We have already seen that its habituation may run very different courses in different people even in the normal condition (3). It is probable, therefore, that the persistence of the glabellar reflex is a nonspecific sign, when it is a result of insufficient habituation of the second component. Pearce and associates found abnormal persistence of the glabellar reflex not only in Parkinsonism but also in certain other conditions, particularly in dementia due to large and diffuse cerebral damage (9). Klawans and Goodwin reported that in such patients the treat-

ment with L-DOPA does not abolish the persistence of the glabellar reflex, which is in contradistinction to the Parkinsonian patients (5).

Even clinical studies alone gave rise to suspicion that the mechanism of persistence may be different in the two conditions.

Our own results however indicate two different mechanisms in Parkinsonian patients themselves, one being the abnormally prolonged first component and the other insufficient habituation of the second component.

It might be interesting to look whether or not these two mechanisms are correlated with any of the aetiological or symptomatic factors of extrapyramidal disorders.

MILAN GREGORIČ

NEUROFIZIOLOŠKA ANALIZA REFLEKSA TREPTANJA KOD PARKINSONIZMA

KRATAK SADRŽAJ

Proučavali smo elektromiografske parametre prve i druge komponente refleksa treptanja kod bolesnika s Parkinsonovim sindromom i kod kontrolne grupe zdravih ljudi. Upotrebljavali smo dva načina stimulacije: regularno ponavljanje električne i mehaničke nadražaje. Prilikom ponavljanja udaraca na glabelu refleksno treptanje kod zdravih ljudi brzo prestaje. Analiza kvantitativnih promena druge EMG-komponente pokazala je kod zdravih ljudi brzo smanjivanje odgovora; osobito kod mehaničke stimulacije — razvijala se habituacija refleksnih odgovora. Kod nekih bolesnika s Parkinsonovim sindromom treptanje prilikom udaraca na glabelu ne prestaje neodređeni period vremena. Kod nekih bolesnika sa tim fenomenom elektromiografska analiza refleksa pokazala je uvećanu prvu komponentu refleksa, uz normalnu habituaciju druge komponente. Kod druge, manje grupe, prva komponenta bila je normalna ili tek malo produžena, dok je habituacija druge komponente prilikom električne i mehaničke stimulacije bila odsutna ili jako usporena. Kod treće grupe bolesnika parametri prve EMG-komponente bili su normalni, a druga komponenta pokazala je normalnu habituaciju, iako je i kod tih bolesnika facijalna muskulatura bila zahvaćena u vidu hipomimije.

Na osnovu naših rezultata zaključujemo da je moguće razlikovati dva mehanizma perzistentnog glabelarnog refleksa kod bolesnika sa Parkinsonovim sindromom: jedan je uvećanje prve komponente refleksa, što i pored normalne habituacije druge komponente dovodi do stalnog refleksnog treptanja, a drugi mehanizam je odsutna ili usporena habituacija druge komponente refleksa.

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DISCUSSION

Kendel: Where have you fixed your accelerometer and what is the weight of this transducer?

Gregorič: We affixed an accelerometer above the eye over the orbicularis oculi muscle. We used mechanical recording only to show that the reflex blinking on tapping the glabella in some Parkinsonian patients really persists. We did not analyse mechanical recording, only the EMG. The instrument weighs about 2 gm.

K. KENDEL AND U. BECK

NOCTURNAL SLEEP IN PATIENTS SUFFERING FROM PARKINSON'S DISEASE UNDER L-DOPA TREATMENT

Experiments on animals with monoaminoacids lead to the conclusion that L-DOPA acts on the sleeping mechanisms, especially the sleep-cycle. Jouvet (1) postulated the existence of neurones with a special sensitivity for monoaminoacids which are responsible for the fundamentally different sleep phases, i. e. the synchronized and desynchronized phases. Wyatt et al. (2) found distinctively decreased REM-phases of sleep but unchanged or slightly increased synchronized sleep in patients treated with-Para-Chlorophenylalanine and L-DOPA. Beside the therapeutic effect on the Parkinson symptoms, an influence on the sleep cycle in these patients is probable under treatment with high dosage of L-DOPA.

15 polygraphical night-sleep EEG registrations were performed in 9 patients suffering from Parkinson's disease before and 12 registrations during L-DOPA therapy of various dosage. We used 4 EEG-registrations by adhesive electrodes and recorded the right and left occipital and the right and left precentral against a vertex electrode. Eye movements, the EMG of musculus mentalis, ECG and respiration were registered too. On the automatic TÖNNIES-EEG-interval-spectrum-analysator, (3) one occipital recording, the eye movements and the EMG were additionally registered. With this method the time-interval between 2 zero points of EEG waves is electronically measured. The trigger-impulse for this time-measuring is corrected in dependence of the amplitude and the reversal points of the single EEG-waves and is not influenced by base-line drives of the EEG. Each of the measured intervals is transformed into the corresponding frequency and is burned into the registration paper as a little black point. Thus the ordinate of the registering paper shows the frequencies of the measured EEG-waves from 1 to 30 Hz. The speed of the paper stripe amounts to 2,5 mm/min. A night-sleep registration of 8 hours is therefore visible on paper stripe which is 120 cm long. Because of this extremely slow paper speed the muscle action potentials which are registered as stripes appear as a black ribbon. The breadth of the black ribbon allows an orientation about the momentary muscle tone. Rapid eye movements are now additionally registered on a third channel as stripes and help diagnosing the REM-phases.

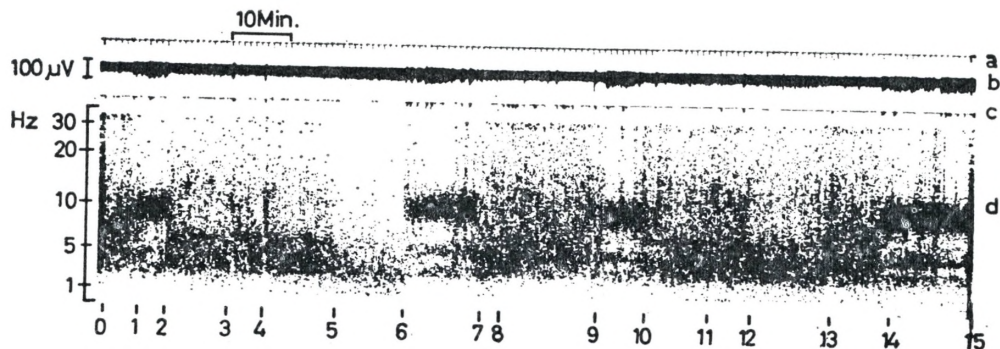


Fig. 1 EISAgramm of a 22 years old healthy student

- a Time (sec)
- b EEG - amplitude
- c Eye movements
- d EEG frequency spectrum

numbers 0 - 15 = different sleep stages

(2 = falling asleep, 8 - 9 REM, 14 = awaking)

When evaluating the night-sleep registrations by means of the EISA-gram each sleep stage can be recognized. In case of uncertainties it can be examined by comparison with the usual EEG-registration. The definition of sleep stages is based upon the international statements by Rechtschaffen and Kales, (4) taking stage I and II as light sleep and stage III and IV as deep sleep.

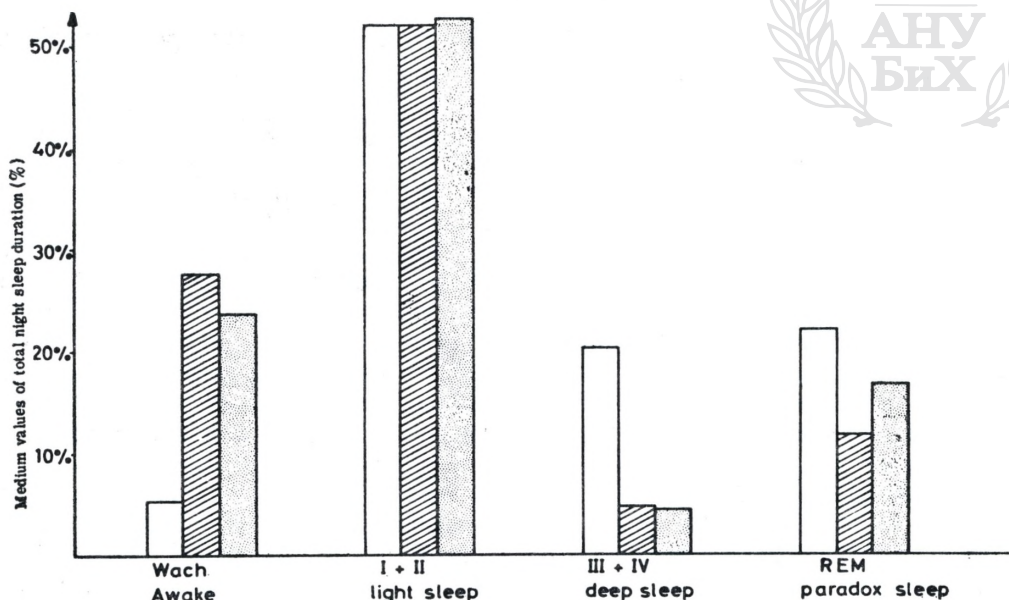


Fig. 2 Sleep stages in patients with Parkinsonism before and during L-DOPA treatment (Percentual medium values of total sleep duration of the different groups)

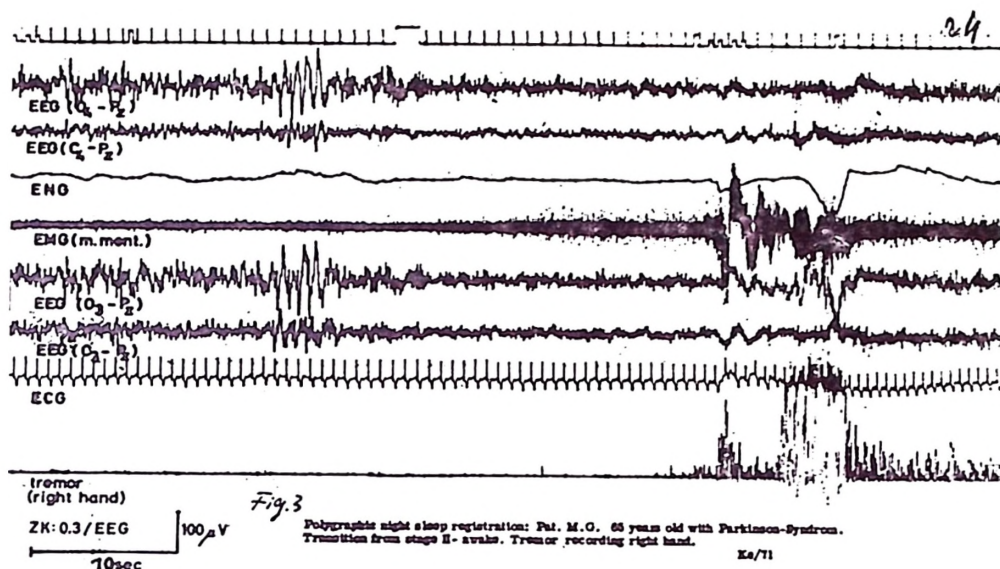
Normal group

Patients with Parkinsonism without L-DOPA treatment

Patients with Parkinsonism during L-DOPA treatment



Ke/71



Beside the quantitative analysis of the sleep stages, the ECG, respiration excursions and tremor were taken into consideration. The influence of L-DOPA on tremor in the different stages of sleep will be especially investigated and compared with the tremor intensity during daytime.

We found that the night sleep of patients suffering from Parkinson's disease without therapy is characterized by abnormalities concerning the duration and distribution of the single stages. Hitherto our investigations show a tendency to normalization of the disturbed night sleep under treatment with L-DOPA. The total duration of sleep and the percentage of REM sleep rise. The sequence of the single sleep stages in Parkinson's disease is disturbed: an investigation of night sleep of healthy young adults shows that the stage REM follows stages I and II (B and C) in 98%, while in Parkinson patients without L-DOPA this percentage is 63% and after L-DOPA treatment 83%. The »REM-Latency« i. e. the duration from the onset of sleep to the first REM stage rises significantly under treatment with L-DOPA.

The comparison of the percentual share of single sleep stages and night waking phases in Parkinson patients under and without L-DOPA treatment shows no difference concerning light sleep. The share of waking phases in patients with Parkinson's disease is considerably higher as in a comparable group of healthy persons. During L-DOPA therapy the portion of the night waking phases decreases a little. In comparison with the normal group it must, however, be mentioned that this group of healthy persons consists of younger subjects. This age-difference is probably responsible for the considerably smaller share of deep sleep in patients with out and during L-DOPA treatment. Though theoretically unexpected, an increase of REM-sleep can be seen in our series during a REM-rebound as described by Wyatt (2) et al. in the second half L-DOPA treatment. A possible explanation of this observation might be of the night after preceding suppression of REM-sleep. This problem needs further investigation.

Only two patients of our series showed an evident extrapyramidal rest tremor. This number is comparatively small as patients with akinesia were preferred for L-DOPA treatment. — The tremor was recorded with a mechanical-electronical-transducer (Accelerometer Burchardt). The transducer was applicated on the hand showing the strongest tremor-intensity.

Both patients with pronounced 4—5 and 5—6/sec rest. tremor in day time showed tremor during night sleep registration only in connection with body movements and appearance of EEG basic rhythm for a few seconds. The tremor frequency was the same as that of the rest tremor in day-time. There was no tremor during synchronized sleep in both subjects. One patient showed tremor with a frequency of 4,5/sec during REM-sleep as in the waking state. The tremor lasted once longer than 20 sec. in a REM-phase.

Summarizing can be stated that night sleep in patients with Parkinson's disease shows deviations from normal sleep. During L-DOPA treatment a tendency to normalization is evident. In patients with tremor a disappearance of tremor in synchronized sleep can be expected. Almost regularly a reappearance of tremor during body movements can be observed. During REM-sleep an activation of tremor may occur.

K. KENDEL I U. BECK

NOĆNI SAN PACIJENATA BOLESNIH OD PARKINSONOVE BOLESTI POD TRETMANOM L-DOPOM

KRATAK SADRŽAJ

Eksperimenti vršeni monoaminokiselinama na životinjama doveli su do zaključka da L-dopa djeluje na mehanizme sna, naročito na ciklusne. Jouvét i saradnici pretpostavili su postojanje neurona sa specijalnim senzitivitetom za monoaminokiseline koji su odgovorni za fundamentalno različite faze sna, tj. sinhronizirane i desinhronizirane faze. Wyatt i saradnici su našli znatan porast REM-faza sna, ali nepromijenjen ili vrlo mali porast sinhroniziranog sna kod pacijenata liječenih parahlorofenilalalinom i L-DOPOM. Pored terapijskog efekta na parkinsonske simptome, vjerovatno uslijed tretmana visokim dozama L-dopa, pojavilo se djelovanje na ciklus sna tih pacijenata.

Izvršen je poligrafski EEG noćnog sna pacijenata sa Parkinsonovom bolešću prije i za vrijeme tretmana različitim doziranjem L-DOPOM. Svi EEG noćnog sna bili su simultano registrirani preko EEG Interval Spectrum Analysatora (EISA, Tonnes). Serije još nisu završene.

Našli smo da je noćni san pacijenata bolesnih od Parkinsonove bolesti bez terapije okarakterisan nepravilnostima u trajanju i raspodjeli *jednostavnih stupnjeva*. Dosada su naša ispitivanja pokazala tendenciju ka normaliziranju nemirnog noćnog sna pod tretmanom L-DOPA; ukupno trajanje sna i postotak REM-sna su povećani. Niz jednostavnih

stupnjeva sna kod Parkinsonskog sindroma je poremećen: ispitivanje noćnog sna zdravih mlađih punoljetnih ljudi pokazuje da stupanj »REM« slijedi stupnjeve I i II (B i C) u 98% slučajeva, dok kod bolesnika sa Parkinsonskom bolešću bez tretmana L-DOPOM taj postotak iznosi 63%, a nakon tretmana L-DOPOM 83%. REM-latentnost« (trajanje od početka sna do prvog REM-stupnja) povećava se znatno prilikom tretmana L-DOPOM.

Pored kvantitativnih analiza stupnjeva sna, registrirani su EKG, pokreti disanja i motorna aktivnost (tremor). Djelovanje L-DOPE na tremor u raznim stupnjevima sna specijalno je istraživano i upoređeno s intenzitetom u toku dana.

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DISCUSSION

Dimitrijević: Could you elaborate a little on mechanism which are influenced by L-DOPA? Are they pharmacological or physiological? I mean often L-DOPA their improves and they become more sleepy because of their physical activity?

Kendel: To 2) The pharmacological or neurophysiological mechanisms on human sleep under high dosage of L-DOPA are unknown. It may be and some experiments support this -that a specific influence on the sleep regulation mechanisms exist. But also we had to take in consideration unspecific components for instance an improvement of akinesia and therefore a better sleep.

NEDO ZEC, MUHAMED GAVRANOVIĆ, DŽELALUDIN KANTARDŽIĆ
AND MIHAJLO JOVANOVIĆ

OUR EXPERIENCES WITH L-DOPA IN THE TREATMENT OF PARKINSON'S SYNDROME

Basal ganglia and similar structures of normal human brain contain the greatest quantity of dopamin (2). However, in patients with Parkinson's disease dopamine is found in very low concentrations in nuclei of extrapyramidal system (nucleus caudatus, putamen, substantia nigra) (6, 8, 1). Biochemical examinations showed that dopamine does not cross the blood-brain barriere but its precursor dihydroxyphenylalanine (DOPA) has this property. Taking this fact in consideration, DOPA was tried in the therapy of Parkinson's disease (5, 9, 4, 3, 7), and most reports show the efficiency of such a treatment. L-DOPA in higher oral doses and with effective results in patients with these extrapyramidal disorders, was used for the first time by Cotzias et al. (4, 5). Similar results were got by Yahr et al. (9). The investigations till now show that this drug gives a great promise in the treatment of this chronic disease of nervous system.

MATERIAL AND METHODS

Our intention was to examine the effects of L-DOPA preparation of the firm »Hoffmann — La Roche AG« Grenzach Baden (caps. à 500 mg) in patients with Parkinson's syndrome.

Our material were the patients with Parkinson's syndrome who were hospitalized at Sarajevo, Neuropsychiatric clinic in 1970. Before the treatment decision the patients were the subjects to adequate clinical (neurological, ophatalmological and internal examination) and laboratory (haemoglobin, red blood picture, reticulocytes, white blood picture, thrombocytes, Heinz-bodies, Coombs'test, ECG, EEG, transaminases, alkaline phosphatase, serum bilirubin, BSP, protrombine time, urine, urea clearance) examinations. Patients with lesions of parenhimatose organs (heart, liver, kidney) were excluded from L-DOPA treatment

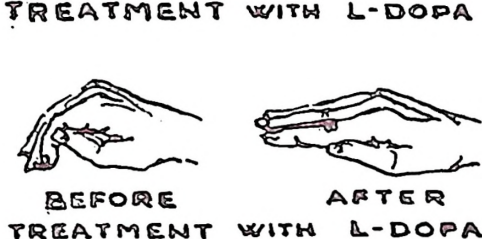
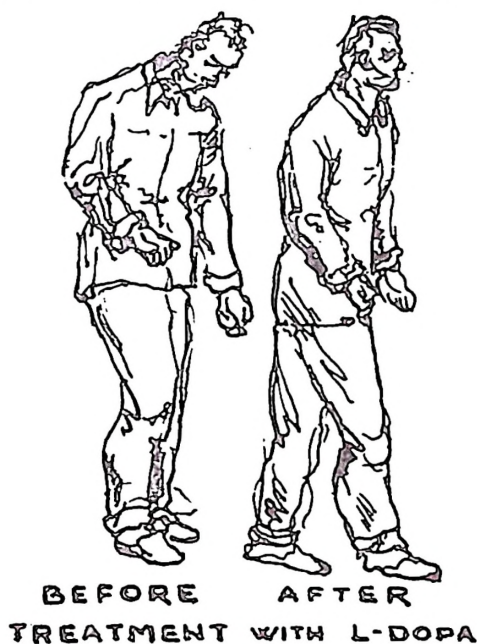
The average duration of the treatment was 90 days. Daily doses were 3—9 g. The beginning was with small doses which successively increased. Clinical and laboratory examinations (as at the beginning)

were performed at the end of the treatment and they were also done during the treatment if it was necessary. The values of pulse, blood pressure and the number of respirations were measured every day.

The evaluation of therapeutic effects was done on the basis of the following parameters: rigidity, tremor, gait and posture, hypomimia, vegetative symptoms (facies oleosa and hypersalivation), handwriting, psychical condition. The results were esteemed as improvement, without effects and decline. The side effects in each patient were observed (nausea, vomiting, hyperkinezia, insomnia, restlessness, depression and complications on parenchymatose organs).

RESULTS

Thirty patients with Parkinson's syndrome were hospitalized. L-DOPA treatment could not be applied in 18 patients because of contraindications (lesions of cardiovascular system, liver and kidney) and so the classic antiparkinson therapy was used. Twelve patients were treated with L-DOPA, five of them with essential Parkinson's disease, six with arteriosclerotic, and one with postencephalitic Parkinsonism.



The results of our examinations are shown in the following tables 1, 2, 3.

T A B L E 1

CASE NO.	INITIALS	AGE	AEIOLOGY	SEX	DURATION (YR)	STEREOTYPIC	DEGL.	RIGIDITY	TREMOR	GAIT AND POSTURE	HYPOKINESIA	VEGET. SYMPT.	HAND-WRITING	BRADY-PSYCHIA	SUBJECTIVE FEELING	SIDE-EFFECTS	MAXIMAL DOSE L-DOPA	FINAL THERAPEUTIC EFFECT
1	A M	46	ES	M	8	+	B	+++	++	++	++	++	++	++	GOOD	HYPERKIN.	9 g	IMPROVEMENT
							A	+	+	+	+	+	+	+				
2	I M	51	ES	M	5	-	B	++	+++	++	++	++	++	++	VERY GOOD	—	6 g	IMPROVEMENT
							A	+	-	+	+	+	-	+				
3	J K	48	ES	M	2	+	B	+++	++	+++	+++	+++	+++	+++	VERY GOOD	—	6 g	IMPROVEMENT
							A	+	+	+	+	+	+	+				
4	S F	55	ES	M	6	+	B	++	+++	++	+	++	++	++	GOOD	INSOMNIA	7.5 g	IMPROVEMENT
							A	+	++	+	+	+	+	+				
5	S F	52	ES	F	5	-	B	+	++	++	+	++	++	++	VERY GOOD	NAUZEA	3 g	IMPROVEMENT
							A	+	++	+	+	+	+	+				
6	P R	76	ART	M	10	-	B	+++	++	+++	+++	+++	+++	+++	BAD	NAUZEA, VOMITING, COR. DECOMP., RESTLESSNESS	3 g	DECLINE
							A	+++	++	+++	+++	+++	+++	+++				
7	H V	63	ART	M	3	-	B	++	+++	++	+	+	++	++	MODERATE	—	3 g	WITHOUT EFFECT
							A	++	+++	++	+	+	++	++				
8	H N	61	ART	M	2	-	B	+++	+++	++	+	+	+++	++	MODERATE	NAUZEA, VOMITING, INSOMNIA	3 g	WITHOUT EFFECT
							A	+++	+++	++	+	+	+++	++				
9	P G	75	ART	M	7	-	B	+++	+++	+++	+++	+++	+++	+++	VERY GOOD	NAUZEA, VOMITING	6 g	IMPROVEMENT
							A	+	++	+	+	+	+	-				
10	E K	68	ART	M	7	-	B	+++	+++	+++	+++	+++	+++	+++	VERY GOOD	—	4 g	IMPROVEMENT
							A	+	++	+	+	+	+	+				
11	M M	60	ART	F	2	-	B	++	++	++	++	++	++	++	VERY GOOD	—	7.5 g	IMPROVEMENT
							A	+	+	+	+	+	+	+				
12	J O	61	PE	F	18	-	B	+++	+++	+++	+++	+++	+++	+++	BAD	RESTLESSNESS, COR. DECOMP., VOMITING, DEPRESSION, INSOMNIA	3 g	DECLINE
							A	+++	+++	+++	+++	+++	+++	+++				

L E G E N D

B	=	BEFORE
A	=	AFTER
+++	=	COMPLETE EXPRESSION OF SYMPTOM
++	=	MODERATE
+	=	MILD
-	=	WITHOUT SYMPTOM
ES	=	ESSENTIAL PARKINSONISM
ART	=	ARTERIOSCLEROTIC PARKINSONISM
PE	=	POSTENCEPHALITIC

Table 2.

	NUMBER OF CASES		
	VERY GOOD IMPROVEMENT	GOOD IMPROVEMENT	WITHOUT EFFECT
RIGIDITY	4	4	4
TREMOR	1	5	6
GAIT AND POSTURE	3	4	5
HANDWRITING	2	6	4
VEGETATIVE SYMPTOMS	3	4	5
PSYCHICAL STATE	2	4	6
HYPOMIMIA	2	5	5
COMPLETE FINAL RESULT	4	4	4

Table 3.

SIDE - EFFECTS	NUMBER OF CASES
1. NAUZEА	5
2. VOMITING	4
3. INSOMNIA	3
4. RESTLESSNESS	2
5. HYPERKINEZIAE	1
6. DEPRESSION	1
7. COR. DECOMP	2

SUMMARY

Thirty patients were admitted at the Neuropsychiatric clinic for the treatment with L-DOPA. All of them suffered from Parkinson's syndrome of different ethyology. After carefull selection (excluding heart diseases, liver damages and lesions of other organs), only 12 patients could underwent prolonged treatment with L-DOPA. Daily dose was 3—9 g. Duration of the treatment was three months.

Results showed good improvement which consists of the decrease of rigidity, salivation, the feeling of bether-being, the improvement of gait and the facial expression. The disappearance of tremor was noticed only at one patient, but the others did not exhibit significant improvement of tremor.

As side-effects are noticed — nauzea and vomiting in five patients, and a few patients exhibited insomnia and restlessness, as well as hyper-

kineziae of temporarily character. The cardiac failure was noticed in two patients, suffering previously of heart disease. Two patients showed increased sexual activity.

We would like to point out the necessity of careful selection of patients, as well as beginning of the treatment in the hospital conditions.

Unfortunately, we did not dispose cocarboxylase inhibitor (MK 485) which decrease significantly the therapeutical doses and in this way makes possible wider use of L-DOPA.

N. ZEC, M. GAVRANOVIĆ, DŽ. KANTARDŽIĆ I M. JOVANOVIĆ

NAŠA ISKUSTVA U TERAPIJI PARKINSONOVOG SINDROMA L-DOPOM

KRATAK SADRŽAJ

U toku 1970. godine hospitalizovano je 30 bolesnika sa Parkinsonovim sindromom na Neuropsihijatrijskoj klinici u Sarajevu radi tretmana preparatom L-DOPA. Kod 18 bolesnika ovaj vid liječenja nije se mogao primijeniti zbog različitih kontraindikacija. L-DOPA je primijenjena kod 12 bolesnika u trajanju od tri mjeseca. Doze su se kretale od 3—9 gr dnevno. Kod 10 bolesnika nastupilo je poboljšanje, a kod 2 pogoršanje. Poboljšanje se sastojalo kod svih u smanjenju rigiditeta muskulature, smanjenju salivacije, opštem popravljanju stajanja, hodanja i izrazu lica. Tremor je potpuno iščezao samo kod jednog bolesnika, a kod drugih nastupilo je neznatno njegovo smanjenje. Prethodno ugašena seksualna potencija vratila se kod dva bolesnika. Pored toga, bolesnici su pokazivali i porast psihičkog energetskog potencijala.

Od sporednih efekata zapazili smo: nauzea i povremeno povraćanje kod 5 bolesnika, kratkotrajna nesanica i uznemirenost kod dva bolesnika, a prolazne hiperkinezije kod jednog bolesnika. Kod 2 bolesnika primijećeni su znaci popuštanja kardiovaskularnog sistema.

Prema našim skromnim iskustvima možemo zaključiti da L-DOPA predstavlja novi veliki napredak u tretmanu parkinsonizma, ali uz prethodnu brižljivu selekciju bolesnika, s obzirom na diferentnost lijeka.

Moramo naglasiti da nismo primjenjivali inhibitor kokarbiksilaze (MK 485), da bismo na taj način smanjili terapeutsku dozu.

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DISCUSSION

Barbeau: I am puzzled by your selection of only 12 patients out of 30 for treatment. This is probably because you are very prudent. The treatment should not be limited by questions of age or previous surgery. The only limiting factor are severe arteriosclerosis (cerebral) and active liver, kidney or heart disease.

Kantardžić: I must say that our patients, who could not undergo the therapy with L-DOPA, were very old almost all of them generalised arteriosclerosis, some of them with hypertension of the second and third degree, some with clear liver and kidney damages. They were in seventh or eight decade of life. The majority of those patients could not walk without help, some couldn't turn from one side to another in bed. There existed social reasons, not only medical, in their hospitalisation. So these were the reasons we did not give L-DOPA to such patients.

We thank to prof Barbeau on these very interesting remarks which we are going to take into consideration in our further investigations with L-DOPA.

Barbeau: One last point about the effect of L-DOPA upon tremor is worth mentioning: the best effect is observed after weeks or even month of administration and is mainly upon the slow 3—5 sec. type of rest tremor. The more rapid attitude or fast tremor occasionally seen in Parkinson disease is often made worse.

Kantardžić: Only one patient showed good improvement of tremor after the treatment with L-DOPA, but we have to point out five patients showed good improvement. Thus means that six out of twelve patients showed a certain degree of improvement of tremor and this is almost equally with results from literature (about fifty per cent).

Dimitrijević: Could I ask you whether the drawings you showed represent your patient-artist himself or another patient.

Kantardžić: The artist made these paintings to show himself, his own good improvement after the treatment with L-DOPA.

Electrophysiological changes



P. J. BERNARD AND A. F. DE SCHAEPPDRYVER

CORTICAL ADRENERGIC AND CHOLINERGIC ACTIVITY AND EXPERIMENTAL TREMOR

It has become clearly established that typical tremorigenic drugs such as tremorine and its active metabolite oxotremorine have a cholinergic, muscarinic type of action (1, 2, 3).

Further it has been demonstrated that tremorine produces a desynchronized alert cortex in the unanesthetized restrained rabbit. The desynchronization is antagonized by atropine (4). This observation may indicate that this EEG effect is a result of its central muscarinic action.

This cortical muscarinic effect prompted us to study the effect of topical administration of muscarinic agents and their antagonists on cortical activity in the unanesthetized restrained rabbit.

We also investigated the effect on cortical activity of several adrenergic agents as well as of their antagonists.

MATERIALS AND METHODS

Analysis of cortical activity was carried out on rabbits restrained in a leather sling.

The animals were trepaned so as to make the motor and sensorimotor cortex accessible. After removal of the dura mater, bipolar silver electrodes were placed on motor and on sensorimotor cortex.

Electrodes were used both for recording and for electrical stimulation.

Electrical stimulation of the motor cortex (usually the right motor cortex) was carried out using 2 msec pulses at a frequency of 30 Hz during 5 seconds. This intermediate frequency stimulation provokes a self-sustained response lasting beyond the stimulus. Activity of the cortex was gauged from the threshold and from the duration of the thus evoked after-discharge.

Topical application was then performed by means of drug-soaked filter paper pledgets. Pledgets measured 2 x 2 mm and weight absorbed averaged 1.5 mg. Drugs were used in a 1% solution.

RESULTS

Topical application of acetylcholine with eserine provokes an inhibition of the after-discharge. The after-discharge is shortened at the treated site and threshold level is increased.

Slide 1 shows the normal response to an electrical stimulation of the right motor cortex.

Slide 2 shows the after-discharge after topical application of acetylcholine with eserine.

This inhibitory effect may be classified as muscarinic as it is reversed by subsequent application of atropine or scopolamine.

Electrical stimulation after topical application of atropine or scopolamine is followed by a lengthening of the after-discharge duration. This is shown in slide 3.

At the site treated with atropine characteristic spikes of high amplitude appear. Similar effects are seen after systemic administration of atropine (5).

Rather surprisingly these spikes are not seen after scopolamine application.

Local anesthesia cannot be considered as a possible basis for the effect of atropine and scopolamine. Xylocaine, when applied topically was found, as can be expected, to inhibit cortical activity. These findings are in accordance with the findings of Krnjevic (6) who concludes that the local anesthetic actions of atropine and of scopolamine on cortical units are transient, lasting only a few seconds.

A further indication of the muscarinic nature of the observed inhibition of cortical after-discharge is provided by the results of topical application of nicotine.

Nicotine applied topically, in contrast with acetylcholine plus eserine, provokes an increase in after-discharge duration which may be reversed by hexamethonium (slide 4).

The effect on cortical activity of adrenergic transmitters and precursors was investigated next.

Topically applied noradrenaline provokes an increase in after-discharge duration. On the average after-discharge duration is doubled.

Topically applied dopamine provokes an increase in after-discharge duration or a lowering of after-discharge threshold.

L-DOPA when topically applied provokes a lowering of after-discharge threshold or an increase of after-discharge duration. Noteworthy is the observation that the increase in after-discharge duration is of higher magnitude than the increase observed after either noradrenaline or dopamine application. In two experiments lowering of threshold and increase of after-discharge duration were observed simultaneously. This was never observed after either noradrenaline or dopamine.

The effect of topical application of adrenergic blocking agents was then investigated.

Phenoxybenzamine, phentolamine, priscoiline and dihydroergotamine do not influence after-discharge threshold or duration.

Beta-blocking agents in contrast with alpha-blockers provoke an increase in after-discharge threshold. Both propranolol, a drug with marked local anesthetic properties and sotalol (MJ 1999), a beta-blocker without local anesthetic properties were used with comparable results.

Finally we should like to report on typical changes in the electroencephalographic pattern at the treated site.

When the treated site is the motor cortex these electrographic changes correspond with involuntary movements of the contralateral limbs. Such changes were observed after application of atropine (slide 5), acetyl-choline plus eserine (slide 6), phenoxybenzamine and other alpha-blockers (slide 7) and propranolol (slide 8).

To conclude, our observations may be summarized as follows (slide 9).

Acetyl-choline (applied with eserine) strongly inhibits cortical activity as gauged from the response to electrical stimulation.

This inhibitory muscarinic action may be reversed by atropine.

Cortical reactivity is also inhibited by beta-blockers.

Cortical after-discharge is facilitated by topical application of atropine and of noradrenaline, dopamine and L-DOPA.

The fact that L-DOPA is more potent than either noradrenaline or dopamine may be an indication that L-DOPA has a pharmacological action of its own. This direct cortical action of L-DOPA may be an important mode of action of this drug, which at present is being extensively investigated for its therapeutic value in the treatment of Parkinson's disease.

Involuntary movements of the limbs may be observed after topical application to the motor cortex of drugs having widely different pharmacological properties.

P. J. BERNARD I A. F. DE SCHAEPPDRYVER

KORTIKALNA ADRENERGIČKA I HOLINERGIČKA AKTIVNOST I EKSPERIMENTALNI TREMOR

KRATAK SADRŽAJ

Uticaj supstancâ na osjetljivost kortikalnih ćelija može se ispitivati promatranjem njihovog efekta na kortikalni prag poslije ispražnjenja i trajanja. Acetilholin lokalno apliciran sa ezerinom snažno inhibira kortikalnu osjetljivost, kako je izmjereno iz odgovora na električnu stimulaciju. Ova inhibitorna muskarinska aktivnost može se povratiti atropinom i skopolaminom. Kortikalna osjetljivost je također inhibirana sa beta-blokerima (propranolol i sotalol). Kortikalno poslije — ispražnjenje je olakšano poslije lokalne aplikacije atropina i nor-adrenalina, dopamina i L-DOPA. L-DOPA je mnogo jači nego i noradrenalin ili dopamin u olakšavanju kortikalno poslije ispražnjenja.

Ovo zapažanje može značiti da L-DOPA ima direktnu farmakološku aktivnost. Direktna kortikalna aktivnost L-DOPA može biti jedan važan oblik aktivnosti ove supstance koji je sada u intenzivnim istraživanjima na planu terapijske važnosti u tretmanu Parkinsonove bolesti.

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A. STRUPPLER, C. H. LUCKING, F. ERBEL AND W. REISS

RHYTHMICAL ACTIVITY IN THALAMIC AND SUBTHALAMIC STRUCTURES

During stereotactic interventions in different types of Tremor we tried to analyse electrophysiologically subcortical motor structures in order to elaborate functional topographic Dates. We used a precorony borewhole, the diameter of the leading cannula was 1 mm, the electrode was insulated up to the tip of 5—10 μ in diameter, the recording was unipolar. Subcortical activity was first preamplified, then recorded on type-recorder and following the operation confirmed by means of a direct-recorder. Tremor activity in the muscle of the limbs (EMG), was recorded by flexible wire electrodes.

I should like to show you the activity in thalamic and subthalamic structures simultaneously with the tremor activity in the limbs: when the electrode reaches the thalamus, spontaneous activity occurs all of a sudden, as you know. We first pass the dorsal oral nucleus of the thalamus in our approach before we reach the ventro-oral nucleus.

Increase of rhythmic tremor-activity can be provoked by letting the patient count down, as seen in slide 1. I should like to emphasize that rhythmic activity occurs in this nucleus before tremor activity in the flexors of the forearm can be detected. The following slide Nr. 2 demonstrates once more the time-relationship between the peripheral tremor and central rhythmic activity, with faster sweep. Whereas figure 1 showed central rhythmic activity before peripheral activation in the flexor muscle we can observe in slide 3 from the Vop central rhythmic activity that is continuing even though the arm-tremors is inhibited by passive manouvre. The proof that we are dealing with cell-activity is this typical »dying cell-activity«, as you see. This lesion can be due to movement of the brain by respiration or pulsation. With faster sweep, you may see in slide 4 that passive inhibition of arm-tremor does not influence that frequency or central rhythmic activity but recruitment within the single bursts.

Passing from thalamus to subthalamus we find a remarkable reduction in spontaneous activity, — in contrast to the approach to the thalamus, as seen in slide 5. At first (— 2,5) we don't find any rhythmic activity, but further penetrating on to — 7,5-approximately the lateral border of the nucl. ruber — rhythmic activity reoccurs, but the frequency is different from the original one before. On the other hand we can observe

rhythmical activity in subthalamic regions with the same frequency as in peripheral tremor as seen in slide 7 in the position — 4, the borderline between the prelemniscal radiation and the zona incerta. Further penetration often results in a complete tremor — stop in the arm, as we will see later; but in that case we usually will find rhythmic central activity with the frequency of the original tremor (8). This indicates that the electrode is located within the dentato thalamic tracts, laterally from the nucleus ruber. Here we are very close to the lemniscus medialis, where evoked potentials can be recorded but not as easily as in the lemniscus itself as well as in the sensory relay nuclei of the thalamus. These evoked potentials seem to originate from joint. Receptors as seen in slide 9 and 10. The identification of the lemniscus can further be achieved by stimulation: sensory sensation often provoke nociceptive motor-responses (i. e. reflexes). (Slide 11). Stimulation in position — 2, — approximately the prelemniscal radiation — evokes only a minimal motor-response: the latter will increase with further penetrating into the lemniscus medialis (position — 4 — 8) often resulting in rhythmic nociceptive responses, the sensory as well as motor effects being dependant upon the intensity as well as frequency of the stimulation (Slide 12).

Rhythmical activity in subthalamic structures seems to play an important role in the peripheral tremor. This will be further confirmed by the fact, that penetration of the electrode alone can cause a remarkable modification of the tremor in the limb. (Slide 13).

We should like to emphasize that we are able to produce this remarkable reduction or even complete stop of the tremor from a very discrete region i. e. the dentato-thalamic tracts predominantly. It seems reasonable to conclude that this system plays an important role in the genesis of tremor, but this problem should still be further discussed. Our recordings seem to confirm the morphological concept especially of Hassler, indicating dentato-thalamic afferents to the Vop since we were able to record rhythmic activity not only in Vop but also in the subthalamus. It therefore seems reasonable to assume that not only the Vop plays an important role in the tremor genesis but we have also to take into account the cerebellar influences upon this nucleus.

A. STRUPPLER, C. H. LUCKING, F. ERBEL I W. REISS

RITMIČKA AKTIVNOST U TALAMUSU I SUPTALAMIČKIM STRUKTURAMA

KRATAK SADRŽAJ

Spontana izazvana aktivnost u raznim supkortikalnim strukturama proučavana je tokom stereotaktičkog tretmana ekstrapiramidalnih sindroma. Kod Parkinsonove bolesti ritmički potencijal iste učestalosti, kao Parkinsonov tremor, registrovani su manje ili više konstantno u predjelima talamusa i suptalamusa.

Razmotrene su sljedeće tačke:

1. odnos između perifernog tremora i ritmičke aktivnosti talamusa i supotalamusa;
2. odnos između ritmičke aktivnosti u različitim nukleusima predjela talamusa i supotalamusa.

DISCUSSION

Jušić: Is there any correlation between the rhythmic activity in subthalamic structures and nociceptive reflex?

Struppler: When you stimulate the med. lemniscal fibre with increasing stimuli firsts you will produce a sensory sensation in the patient without any motor response. With greater stimuli often there becomes a motor response dependent of the pain-sensation, in the leig, i. e. a nociceptive reflex response.

Bruggencate: Since the voltage for a threshold stimulation is so very much depended on a given electrode (e. g. resistance, polarisation) I wonder if you also measured the current for the threshold stimulation. I feel this may help you to compare both results and electrode localisation in different patients.

Struppler: Up to now we have not measured sistematically the role of the stimulus intensivity that means the current intensivity for identifying different motor structures, like N. ruber. Thank you for your remarks, this method will be helpful for us to identify for example the N. ruber system.

Dimitrijević: One dull, stereotypical question. In how many patients you have collected this results you have presented?

Struppler: No, we were n'table to record »silent cells« when the patients was cuonting which was described by Bates. As far as I know, Bates has recorded this types of cells in thalamic structures whereas our recordings was taken especially in subthalamic structures.

S. S. SCHÄFER

THE GAMMA-INNervation OF PRIMARY MUSCLE SPINDLE ENDINGS AS A TRIGGER MECHANISM IN COLD SHIVERING

The typical rhythmicity of the α -motoneurons during a cold shivering tremor originates not before the spinal level. The experience of different authors leads to this result. (Henatsch et al., 1966; Hufschmidt, 1959; Perkins, 1946; Birzis and Hemingway, 1957; Lippold et al., 1959; Meurer et al., 1967; Klusmann and Henatsch, 1969). But the efforts for finding a pacemaker for the rhythmicity of the α -motoneurons at spinal level were up to now disappointing. This has prompted the idea that the rhythmicity might be generated within closed neuronal feedback loops that become unstabilized for some reason. One of the feedback systems most frequently discussed in this respect consists of the extrafusal muscle, the muscle spindle and their primary afferents ending at the α -motoneurons, and the α -motoneurons themselves.

The first figure shows a schematic drawing of this servocontrol system. One can see the skeletal muscle and parallel to the muscle its stretch receptor, the muscle spindle. The muscle spindle for its part possesses its own motor system that the figure represents in a very simplified manner (Barker, 1967; Boyd, 1968). It comes from the γ -motoneurons of the spinal cord then proceeds to the muscle spindles. The sensory afferent fibres of the muscle spindle lead to the spinal cord and terminate at the α -motoneurons of the same muscle. These elements the extrafusal muscle, the muscle spindle, and the α -motoneurons together form a negative feedback system. This servo-loop might possess stability or instability depending on conditions to be explained. During ordinary movement the servo-loop works stable. During a cold shivering, on the other hand, the stable behavior degenerates into instability. Such a change in the behavior of the servo-loop takes place through an intervention of the central nervous system in the servo-loop. The modulating action of the central nervous system can be pointed out at the α -motoneurone level (Stuart et al., 1966 b; Klusmann, 1969; Meurer et al., 1967) as well as at the muscle spindles (Stuart et al., 1966 a; ten Bruggencate et al., 1964; Schäfer, 1969; Henatsch, 1966). Both elements change their properties during cold shivering.

Our interest concentrated on the kind of changes in the properties of the muscle spindle and how far these changes might account for the onset of an instability. If this question is worked out biocybernati-

cally simple mathematics results in the prediction that the muscle spindle advances the instability of the servo-loop if its first and second order differential sensitivities are reduced during cold shivering and/or its proportional sensitivity is enhanced. Because of the limited time we can not discuss the mathematics of these calculations in detail. Only so much might be mentioned that the transfer function of the servo-loop was obtained and that we applied the so-called Hurwitz stability criterion (Oppelt, 1964) to our closed loop system equation to determine the conditions for the instability of the servo-loop.

The decrease in the differential sensitivities and the enhancement in the proportional sensitivity of the muscle spindle that are theoretically required during cold shivering were experimentally examined in the discharge frequencies of primary muscle spindle endings. For this purpose the discharge frequencies of spindle afferents from the gastrocnemius muscle of the cat are recorded in response to ramp stretches of the same muscle. The sensitivities were determined from the discharge patterns before, during, and after cold shivering. The cold shivering tremor was obtained by simply cooling the cats. Figure two shows a discharge pattern of a primary ending during a ramp stretch. The pointed curve represents the discharge frequencies of the spindle with the individual symbols indicating the reciprocal intervals between neighbouring spikes. The upper curve demonstrates the stimulus used to stretch the muscle from a resting length with a definite velocity to a final value. The frequency of impulses coming from the muscle spindle depends upon the acceleration, velocity, and length of the stretch. The acceleration of the stretch is measured in the discharge pattern by the initial burst marked by the first arrow, A. After the first peak the spindle develops a second peak at the end of the stretch rise and then the discharge rate drops to a maintained new frequency value. The difference between the frequency peak at the end of the stretch rise and the maintained value marked with S is proportional to the velocity of the stretch. This value is the dynamic response, D. Finally the length of the stretch is measured by the static response the maintained frequency marked by the third arrow, S.

For the considerations of the instability we are actually not so much interested in the responses of the muscle spindle but instead in their sensitivities. We can extract the first order differential sensitivity from the dynamic response and the second order differential sensitivity from the acceleration response. The proportional sensitivity can be obtained from the static response.

Figure three shows the determination of these sensitivities as well as their changes during cold shivering tremor. One can see in the diagram a) the acceleration response as a function of the acceleration of the stretch. The values of the resting behavior of the cat are marked by crosses and open circles, whereas the value during cold shivering tremor by closed circles. The curves rise linearly over a wide range. One can see, that the slope of the shivering curve is less. The ascent of the curves corresponds to the second order differential sensitivity. Consequently, the second order differential sensitivity decreases during cold shivering tremor. The diagram b) shows the dynamic response

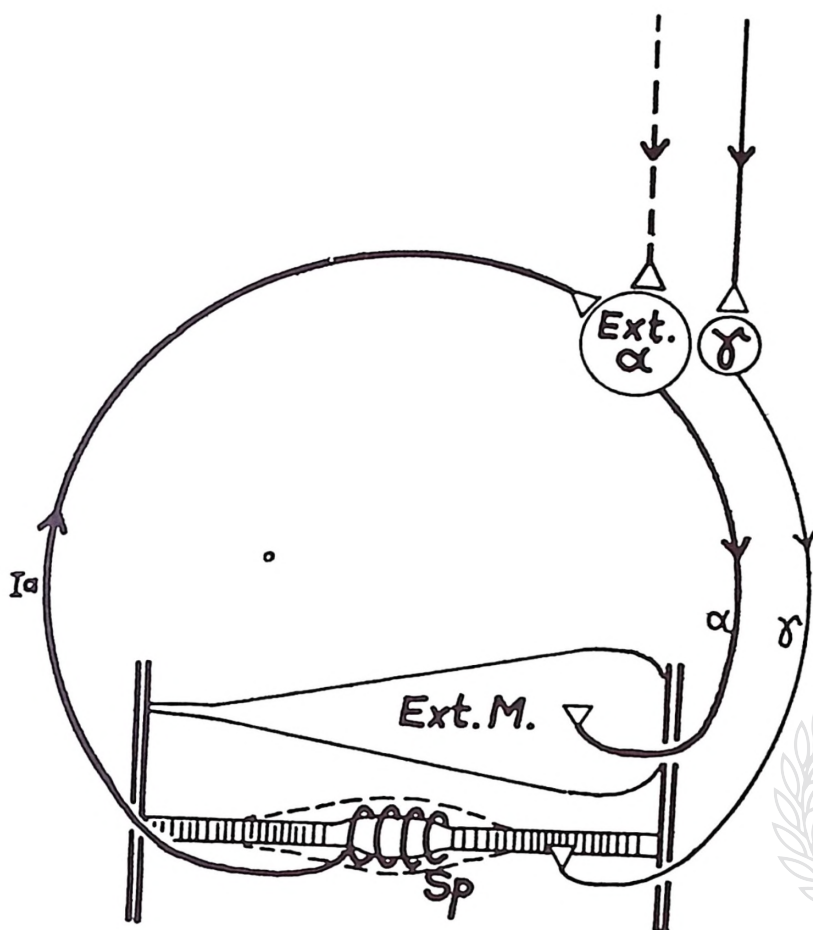


Fig. 1

A simplified reproduction of the stretch reflex feedback system. Ext. M.: extrafusal muscle; ext. α: α-motoneurone; γ: γ-motoneurone; Sp: muscle spindle; Ia: primary afferent fibre. During cold shivering tremor there occurs an instability in the feedback system.

as a function of the velocity of stretch. The resting and shiver values are characterised as in a). As one can see, the ascent of the shivering curve decreases here as well. Because that ascent of these curves corresponds to the first order differential sensitivity, this sensitivity, too, declines during cold shivering. Thus, both differential sensitivities fulfil the conditions of the cybernetical calculations by which the first and second order differential sensitivity becomes smaller if the muscle spindle participates in the instability of the servo-loop.

The proportional sensitivity is evident from the ascent of the curves of the static response, demonstrated in diagram c). Here the values during resting behavior and cold shivering lie on one curve. There were some spindles that showed an increase in their proportional sensitivity but just the same number of spindles had also a decrease in their sensi-

tivity. So on the whole one can say that the proportional sensitivity does not change during cold shivering. By this behavior the proportional sensitivity does not contribute to the onset of an instability. In this case one would expect the sensitivity to increase during cold shivering. But on the other hand, the proportional sensitivity does not counteract an instability, it just behaves indifferent. By these findings the muscle

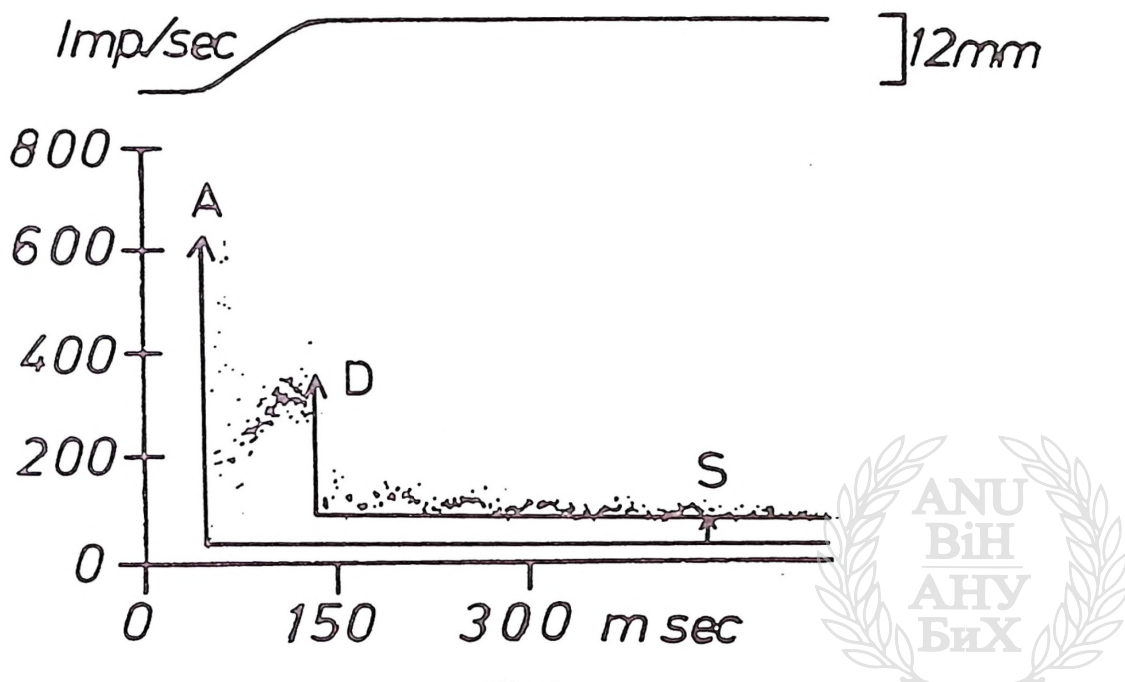


Fig. 2

Response pattern of a primary spindle ending during a ramp stretch to the extrafusal muscle. A: acceleration response; D: dynamic response; S: static response.

spindle does give rise to an instability in the servo-loop extrafusal muscle, muscle spindle, spinal cord by a decrease in their first and second order differential sensitivities.

The change in the slope of the characteristic curves is generated by central γ -inervation of the muscle spindle. The type of central γ -inervation had now to be identified, in order to become acquainted with the activity of the central nervous system which descends during cold shivering and leads to the change in the sensitivities of the muscle spindle.

To this end the fusimotor fibres innervating the muscle spindles were artificially stimulated at the ventral roots and the curves during γ -stimulation were compared with those during cold shivering tremor. Figure four shows in diagram a) the curves of the dynamic response with and without γ -stimulation. As in the afferent system it is customary to distinguish between a static and a dynamics component also in the

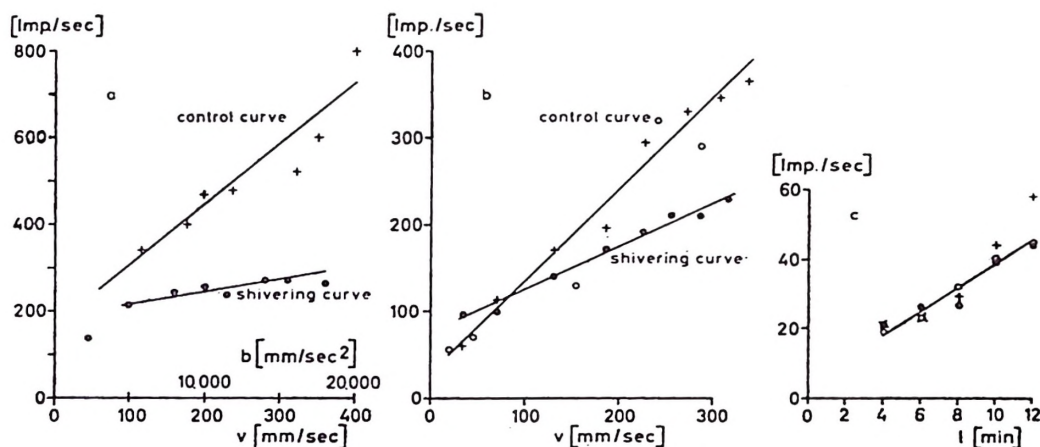


Fig. 3

Characteristic curves of primary spindle endings before (+), during (●) and after (○) cold shivering tremor. a: Acceleration response as a function of the acceleration of stretch. b: Dynamic response as a function of the velocity of stretch. c: Static response as a function of the length of stretch. The slope of the curves shows the sensitivities of the muscle spindle. Note that the differential sensitivities (a, b) decrease during cold shivering tremor.

efferent γ -motor supplies sometimes referred to as γ_1 and γ_2 . Here the effect of the stimulation of a dynamic fusimotor fibre is demonstrated. The behavior of the dynamic response without γ -stimulation is given by the curve marked with crosses. The dynamic response during γ -stimulation is shown by closed triangles. During the dynamic γ -stimulation there occurs a decrease in the slope of the curve as it was the case during cold shivering. Not only the decrease of the slope but also the intersection with the control curve corresponds with the behavior during cold shivering. Since the characteristics during γ -stimulation and during cold shivering show the same course it is safe to say that during cold shivering an enhanced dynamic γ -innervation is present.

The static γ -innervation during cold shivering was read from the behavior of the spontaneous activity of secondary muscle spindle endings. In many experiments they were simultaneously recorded. Here regularly a decrease of the spontaneous activity took place. From this finding (Appelberg et al., 1966; Brown et al., 1967) one is led to conclude that during cold shivering the static γ -innervation is depressed simultaneously from supraspinal levels.

Thus, during cold shivering there occurs a changed fusimotor innervation to the effect that the dynamic innervation is enhanced and static γ -innervation is decreased, which then leads to the described decrease of the differential sensitivities first and second order.

The change in the γ -innervation seems to be essential for the rise of a typical cold shivering tremor. So the enhancement of the dynamic γ -innervation regularly parallels the appearance of the visible tremor (Klussmann, 1969; Henatsch et al., 1966). And the γ -innervation was

changed just before the beginning of a burst of tremor from the supraspinal centres. A few seconds later, directly as a consequence of the changed γ -innervation, one notices the beginning of the tremor. When the tremor comes to an end also the enhanced γ -inner-

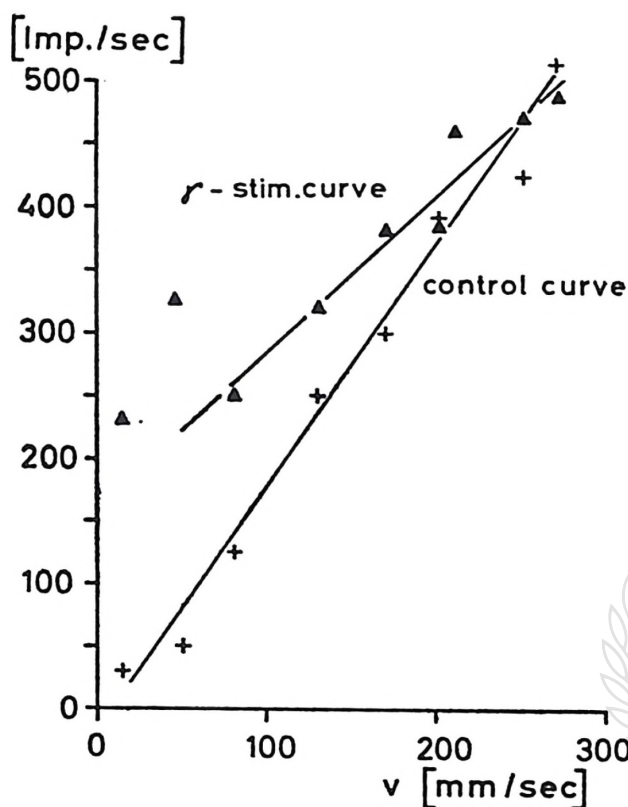


Fig. 4

Characteristic curves of the dynamic response with (▲), and without (+) dynamic γ -stimulation. During dynamic γ -stimulation there occurs a decrease in the slope of the curve and an intersection with the control curve as it happens during cold shivering tremor (Fig. 3b)

vation stops. Moreover, it was possible to establish that in those cases in which the cats showed a pronounced cold shivering tremor with large amplitudes a high dynamic γ -innervation had taken place, whereas a weak tremor with small amplitudes showed only a slightly enhanced γ -innervation. The above given results together with these observations can be interpreted in that way, that by the muscle spindle the servo-loop is brought into the instability.

This statement is in contrast with the interpretation of other investigators (Stuart et al., 1966b; Pierau et al., 1965; Meurer et al., 1967) who claim that the oscillations of a cold shivering tremor are generated only in the spinal cord respectively only by the α -motoneurons. Deaffe-

rentation experiments are quoted as evidence for this idea since after cutting the dorsal roots only the behavior of the α -motoneurons is observed because the muscle spindles are eliminated. And indeed under this experimental condition in some cases a tremor occurs when the cats are cooled. But the tremor differs considerably in its frequency and amplitude from the oscillations of a physiological cold shivering tremor. Consequently, these experiments show that the spinal cord by itself is able to become an unstable control element. But this observation does not imply that the whole feedback system becomes unstable. So it is possible that a feedback loop consisting of two unstable control elements shows a stable control behavior (Opelt, 1964, S. 447). The stable or unstable behavior of the feedback system is dependent on the properties of all control elements forming the feedback system. On the basis of this assumption the postulated contribution of the spinal cord to the instability is not conclusive. However, our results show that the muscle spindle by a decrease in their differential sensitivities renders the feedback system into an instability.

S. S. SCHÄFER

GAMA-INERVACIJA PRIMARNIH VREtenASTIH ZAVRŠETAKA MIŠIĆA KAO OBARAČKI MECHANIZAM HLADNOG TREMORA

KRATAK SADRŽAJ

Prilikom drhtanja usljed hladnoće uočena je, pored promjene alfa-inervacija, i promjena gama-inervacija. Gama-inervacija za vrijeme drhtanja usljed hladnoće sastoji se od povišene aktivnosti dinamičkih gama-neurona i od povećanja aktivnosti statičkih gama-neurona. Ova vrsta promjene gama-inervacije rezultira povećanjem senzitiviteta primarnih vretenastih završetaka mišića u odnosu i na brzinu i na akceleraciju.

Prema biokibernetičkim razmatranjima, ova povećanja senzitiviteta mogu dovesti do instabiliteta (= tremor) u feedback-sistemu (ekstrafuzalni muskulo-muskularni vretenasto-kičmeni stub). Prema tome, za drhtanje usljed hladnoće dovoljni su impulsi iz nivoa kičmenog stuba.

Dalje, iz eksperimentalnog i biokibernetičkog dokaza slijedilo bi da su vretenasti završeci mišića preko porasta senzibiliteta ti koji pro-uzrokuju drhtanje usljed hladnoće. Zbog toga što vrijednost povećanja senzitiviteta vretenastih završetaka mišića zavisi od istovremene gama-inervacije iz viših centara, ti centri mogu započeti, modificirati ili završiti periferni mehanizam drhtanja usljed hladnoće.

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DISCUSSION

Jurna: Dr Schäfer, I had the feeling that you tried to discard the spinal cord as an important rife for tremor generation. Does that mean that could influences the spindles discharge peripheraly? Has it something to do with changes in the initiation of generator poteties? If not, then the spinal cord is still necessary for tremor generation?

Schäfer: Changes in the behavior of the alpha-motoneurons. I think are necessary for the rise of a cold shivering tremor. But on the other hand it is not possible to say that only the alpha motoneurons are responsible for the generation of shivering tremor. For, if the cold shivering tremor generates in the feedback loop than all control element forming the nervo system have the same importance for the rise of an instability. That means that there is no priority between the spinal cord and the muscle spindle for the generation of a cold shivering tremor.

Stern: Have you tried in your experiments to give Atropine or Holinomimetica? Also it would be interesting to know how does succinylcholin act.

Schäfer: I only looked for the physiological cold shivering tremor, so I have not had treatments with drugs. But a test with succinylcholin would be very interesting for it enhances the dynamic fusimotor effect at the muscle spindle discharge frequencis. Perhaps, it is possible to test by this way the idea that the muscle spindle and the changes in their sensitivities are important for the generation of the tremor at spinal level.

Bruggencate: Dr Schäfer, what is your definition of dynamic gamma-stimulation? Although obviously both with dynamic gamma-stimulation and cold shivering the slope of your curves was diminished, in cold shivering the whole curve was very much below the control. Can this also be explained with an increase in dynamic gamma activity? Did you also record from filaments efferent gamma-activity under the condition of cold shivering.

Schäfer: The dynamic gamma-stimulation is difined by an increase of dynamic response during the stimulation.

During the cold shivering tremor occurs not only an increase in the dynamic but also decrease of the static gamma innervation. A decrease of the static gamma innervation results in a decrease of the slope of the dynamic response (unpublished results) so the marked diminution of the slope of the shivering nerve results by an addition of the effects of the changes in the static and dynamic gamma innervation. We did not recorded the gamma-activity from ventral root filaments for the sorte of gamma innervation is more precisely read from the changes in the discharge frequencis of the spindle efferents.

SZ. TÓTH, É. OROSZ AND J. JUHASZ

THE EFFECT OF SOME PARKINSONOGEN DRUGS ON THE CEREBRAL ELECTRICAL ACTIVITY OF THE CAT.

A great number of drugs are known which in animal experiments and in clinical use, as by effect, produce hypokinetic rigid syndrom. Without looking at the experimental side of the question, the problem is very important because it emphasizes, that Parkinson syndrom is a rather general type of reaction of the nervous system or moreover the motorium, which can be elicited by different actions. This contradicts the presumption of Poskanzer and Schwab (1963), that Parkinsonism is in the process of disappearing, because epidemical encephalitis epidemia did not occur since World War I. We must range the Parkinson syndrom — not in its mechanism, but in its nature — as epilepsy or as hyperkinetic syndrom, which occurs with a certain type of injury of the nervous system, quite independently from the reason that causes the injury or the functional disturbance.

It is well known that the electrical signs of the Parkinson disease are very scarce, not specific or can not be observed at all, (Hughes, 1966) mainly when not taking into consideration attempts of activation tests. (Tóth — Tomka, 1968). Therefore, it is the more interesting that parkinsonogen neurolept drugs change the cerebral electrical activity, and so enable the investigation of the effect producing Parkinson syndrom, by a routine clinical method. These considerations induce us to carry out model investigations of some neurolept drugs in animal experiments.

21 curarized and respired cats were used for the experiment. 3 pairs of electrodes were placed into the skull on both sides. The deep electrodes were introduced by stereotactic way into the thalamus (n. V—L), pallidum and the mesencephalic reticular system on both sides.

The cerebral activity and evoked potentials gained by sound and light stimuli were registered by a 16-channel ELEMA EEG apparatus. The femoral arterial pressure was also continuously registered. Drugs were administered intravenously. 2,5 mg of Dehydrobenzperidol (DHBP), 1 mg of Rausedyl (Reserpine) and 1,25 mg of Haloperidol were administered repeatedly. (The largest doses of DHBP contained 4 x 7,5 mg, of Rausedyl 5 x 1 mg, and of Haloperidol 4 x 1,25 mg.).

We investigated the action of the combination of the three drugs mentioned and also the combination with pentamethylentetrazol.

According to our experiences the three drugs change the cerebral electrical activity in the same way but to different degrees. With all the three drugs, just after the injection, first the activity is desynchronized and the beta activity dominates. This activity quickly disappears and gives way to an extensive alpha-theta activity. Frequently the alpha, theta waves occur in short bursts with high amplitudes. (Fig. 1. a), b), c). (Within these bursts many sharp, spikelike waves occur diffusely. (Fig. 2. a), b). (The bursts consist — mainly after Haloperidol — of sinusoid

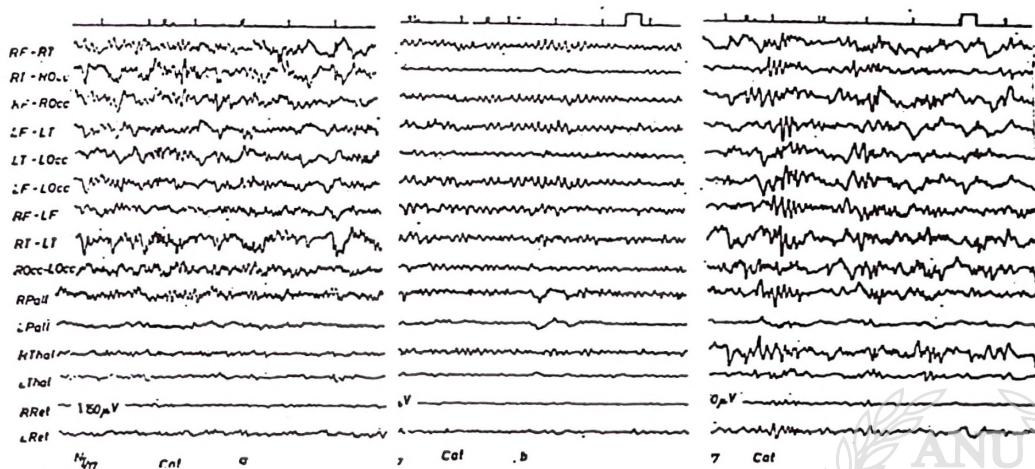


Fig. 1

Cat N I/17

- a) Activity without medication
- b) After 7,5 mg DHBP: slowing down and stable alpha activity
- c) After 2 x 7,5 mg DHBP: alpha activity in short groups

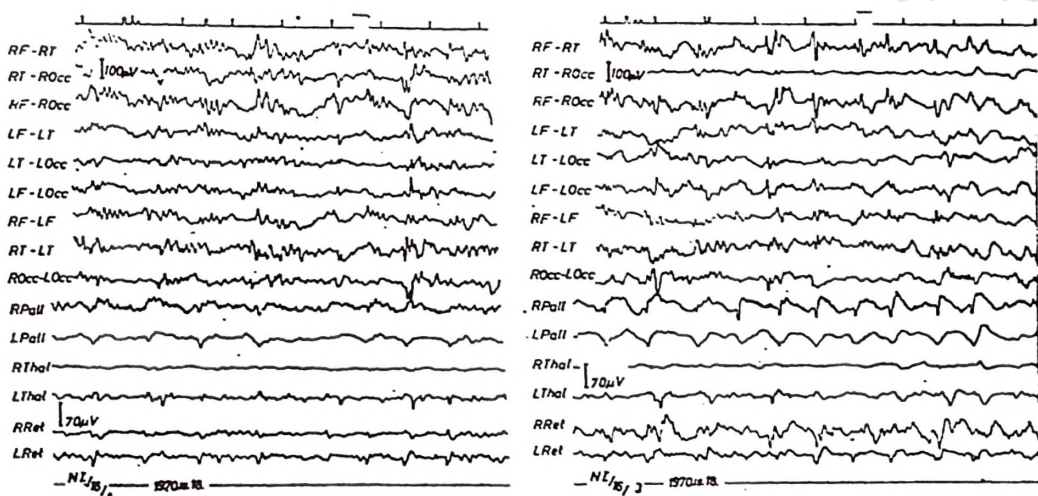


Fig. 2

Cat N I/16

- a) Unmedicated state
- b) After 7,5 mg DHBP: sharp, spikelike waves appear

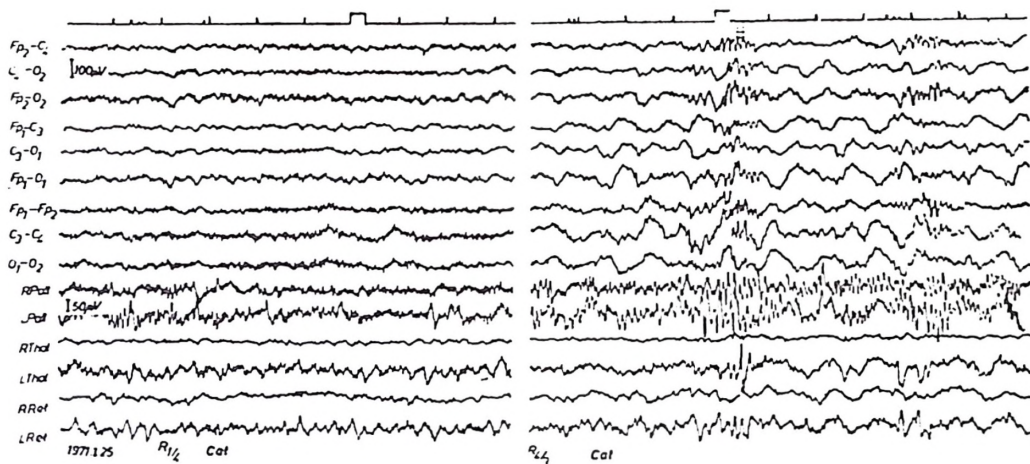


Fig. 3

Cat R/4.

a) Unmedicated state.

b) After 1,25 mg Haloperidol: general slowing down and sinusoid alfa-theta groups appear. Increasing activity of the deep electrodes.

theta-delta waves combined with sharp waves. (Fig. 3. a), b). With higher doses the electrical picture does not change essentially, but more and more slow waves appear diffusely in the activity. When no more drugs are given, the changed activity prevails for hours. Under the effect of rising doses the activity of the thalamus, pallidum and reticular substance is more and more expressed. This is particularly remarkable in the thalamic activity because without drugs this activity is the

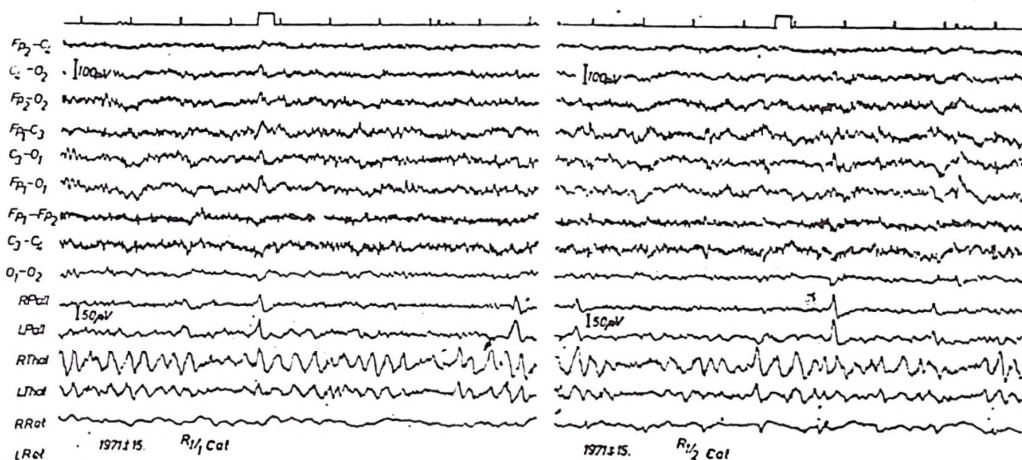


Fig. 4

Cat R/1.

a) Unmedicated state. During stimulation by light no evoked potentials.

b) By sound stimulation evoked potentials can be seen on the reticular electrodes and sporadically on the cortical ones too.

poorest. Giving Rausedyl (Reserpine) Haloperidol and DHBP in combination or one after the other their effect on the cerebral electrical activity is more marked than when given only one of them repeatedly. (Fig. 4. a), b), and Fig. 5. a), b).

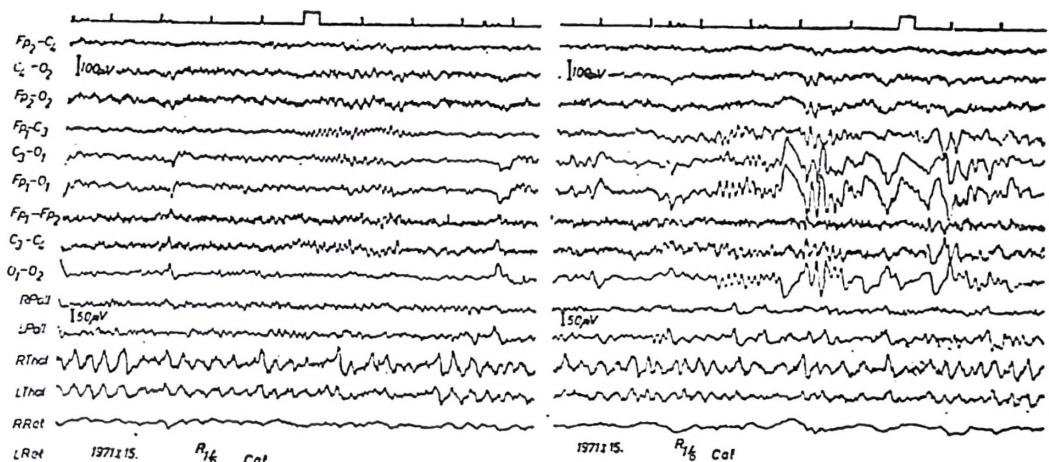


Fig. 5

Cat R/1.

- After 2 x 0,5 mg Rausedyl (Reserpine): sinusoid alfa activity
- After 2 x 0,5 mg Rausedyl + 6,25 mg DHBP: sinusoid groups of alfa-theta waves can be seen with high amplitudes.

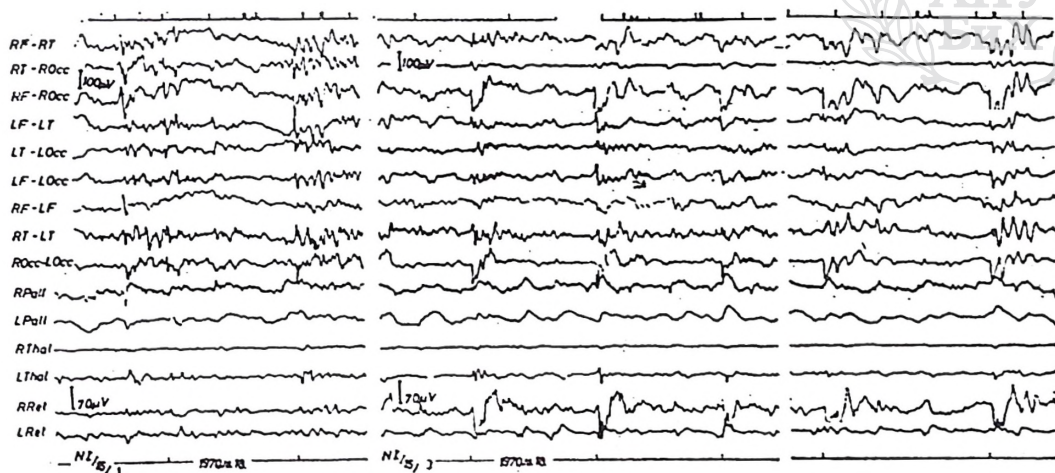


Fig. 6

Cat N I/16

- Unmedicated state. Definite responses can not be seen on light stimuli.
- After 7,5 mg DHBP: definite responses, in accordance with the stimuli, evoked by light.
- After 7,5 mg DHBP: marked multiple discharges evoked by sound stimuli. (In unmedicated state response was not to be gained by sound).

After neurolept premedication, the irritative signs evoked by pentamethylen-tetrazol are more intensive and more prevalent. This effect is most marked after DHBP, less expressed after Haloperidol and least notable after Rausedyl. After DHBP the effect may reach such intensity that repeated electrical seizures appear, for a prolonged time.

Evoked potentials of sound and light stimuli are also changed by the effect of DHBP, Rausedyl and Haloperidol. After the administration of the three drugs the amplitudes of the evoked potentials and the number of after-discharges increase. The evoked response is becoming stable, or more exactly, the responses follow the stimuli more systematically than without medication. (Fig. 4. a), b), Fig. 6. a), b), c), Fig. 7. a), b), Fig. 8. a), b.)

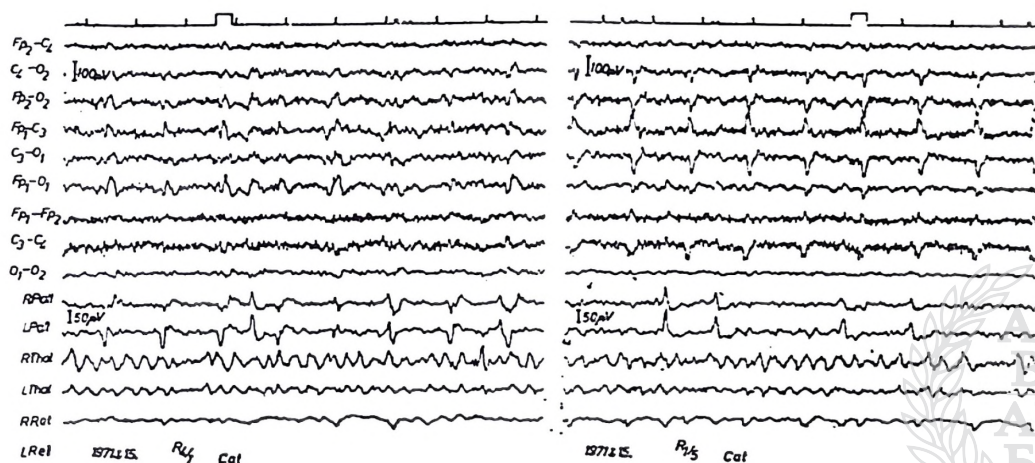


Fig. 7

Cat R/1.

- a) After 0,5 mg Rausedyl (Reserpine), stimulation by light: pronounced evoked potentials on the pallidar and cortical electrodes.
 - b) After 0,5 mg Rausedyl, sound stimulation: evoked potentials on the reticular and cortical electrodes.
- (The unmedicated and stimulated state see in Fig. 4 a) and b).)

In literature many references can be found in connection with the cerebral electrical changes observed also by us. According to Raoul Jeri (1956) Reserpine activates the petit mal and grand mal activity. Rinaldi and Himwitch (1953), observed the activation of the reticular substance due to Reserpine. Sigg and Schneider's (1957) examinations are similar to those mentioned above. According to their observations Reserpine activates convulsive activity in the rhinencephalon. Gonnard and Co. (1956) mention the convulsant supporting effect of Reserpine. Many authors are rather interested to find relation between the tranquillizing effect and the EEG signs of the said drugs. (Liberson, 1956, Monnier and Gangloff 1956, Ekiert and coll. 1959, Wilson and coll. 1964).

Some authors attribute to Reserpine and Haloperidol once an inhibitory, at other times excitatory effect, depending on the basic function of the brain. (Kamenskaja and coll, 1965, Geyer and coll. 1967). No

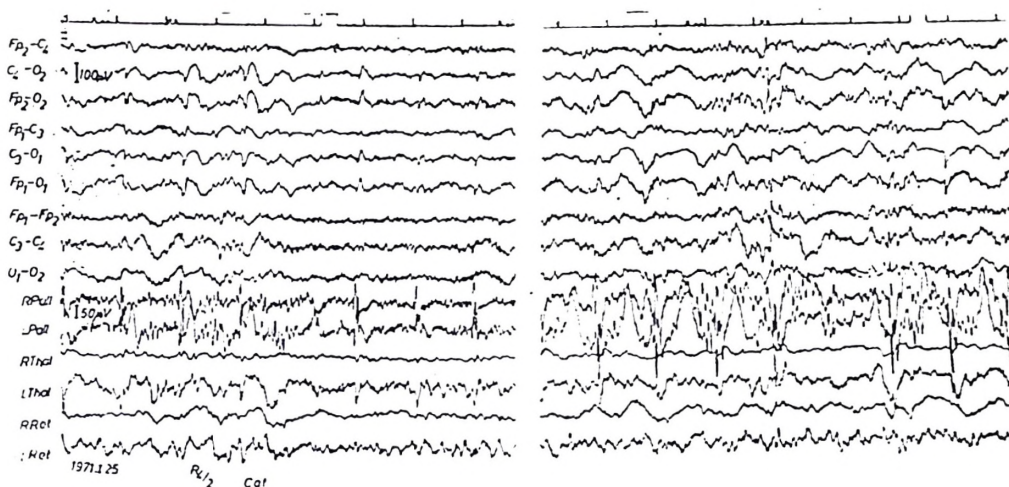


Fig. 8

- a) Unmedicated state. Evoked potentials by light stimuli on the pallidar and thalamic electrodes, sporadically on the cortical ones too.
 b) After 2 x 1,25 mg Haloperidol: The evoked potentials are more marked both on the cortical and the deep electrodes.

data relating to the effect of DHBP on the cerebral electrical activity can be found, but our investigations carried out in neuroleptanalgesia (Orosz and coll, Tóth and coll.) reinforce our results described above. We did not find any literary contributions which deal with the parkinsonogenic effect of neurolept drugs in connection with EEG signs either.

The interpretation of this interrelation is by no means easy and can only be approached with due care.

No doubt however, that EEG and deep electrode examinations of patients with Parkinson syndrom may indicate hypofunction, — or perhaps the relative hyperfunction of certain areas — and the decrease of reactivity.

In contrast to the above, the change of the electrical activity of the brain, due to neurolept drugs is indicative of hyperfunction, irritative activity and increased reactivity, in general.

That seems to suggest that the functional disorder leading to dyskinesia takes place on a different level in Parkinsonism than in conditions elicited by the effect of neurolept drugs.

It is worth mentioning that the stable and extensive alfa activity is observable both in Parkinsonism and in condition due to neurolept drugs.

Evoked responses gained under the effect of Rausedyl (Reserpine), Haloperidol and DHBP occur regularly uniformly and with higher amplitudes. This should allow for the conclusion that in this condition selective control function decrease.

EFEKT PARKINSONSKIH LIJEKOVA NA ELEKTRIČNU AKTIVNOST MOZGA

KRATAK SADRŽAJ

U prethodnim ispitivanjima pokušali smo da pronađemo metode koje bi nam mogle pružiti priliku da procijenimo operativni kapacitet pacijenata sa Parkinsonovom bolešću. Ispitivanja smo bazirali na pretpostavci da se reaktivnost mozga mora izmijeniti tokom bolesti i da mogu da postoje korelacije između reakcije na odgovarajuće opterećenje i reakcije na hirurške zahvate.

U sadašnjim ispitivanjima analizirali smo u vezi sa naprijed navedenim model-uslove i razvijenu reaktivnost mozga koju su izazvali parkinsonski lijekovi.

U toku eksperimenata mačkama smo dali povećane količine dehydro-benz-perydola, rauwolfia derivata i Haloperidol. Proučavali smo promjene električne aktivnosti mozga i izazvane reakcije na svjetlosne i zvučne stimulanse. Primijetili smo da je EEG pacijenata sa Parkinsonovom bolešću sličan EEG-u koji su prouzrokovali parkinsonski lijekovi. Reakcija na svjetlosne i zvučne stimulanse mijenja se pod uticajem parkinsonskih lijekova (isto tako i reakcije na električne stimulanse, kako smo vidjeli kod stereotaktičnih hirurških zahvata). Intenzitet izazvanih reakcija raste sa povećanjem količine lijekova, što nam može pokazati hiperaktivnost stimuliranog sistema, ali i njegovu nestabilnost. U stupnju izazvanih reakcija na korteks i supkortikalne ganglije (talamus, n. ventrolateralis, pallidum, substantia reticularis) postoji promjena uglavnom u pravcu supkortikalnih ganglija.

Na osnovu naših rezultata pokušali smo da analiziramo mehanizam djelovanja parkinsonskih lijekova. Uporedili smo efekt Parkinsonovog sindroma na ljudskom kliničkom materijalu sa eksperimentalnim parkinsonskim efektom.

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A. JUŠIĆ, S. DOGAN AND N. FRONJEK

THE EFFECTS OF LOW FREQUENCY MUSCULAR STIMULATION ON EXTRAPYRAMIDAL SYMPTOMS

Since 1968 patients with Parkinson's syndrome have been treated with the method of low frequency muscular stimulation developed by Hufschmidt (1) for treatment of spasticity.

A total of 30 patients, aged between 34 and 73 were treated with this method. All these patients were examined by necessary clinical, laboratory and X-ray analysis.

Stimulation was performed by the stimulator »Spasmotron« using parameters proposed by Hufschmidt (1). Two pairs of electrodes were located on each antagonistic or symmetric muscular group. The procedure regularly started with the stimulation of the trapezius-deltoides, biceps-triceps, extensors/flexors of the hand and fingers. In the lower extremities the lumbar musculature or biceps femoris was stimulated alternately with m. vastus medialis — m. gluteus medius of the contralateral side. To minimize speech impairment and pseudohypersalivation due to swallowing insufficiency, the laryngeal region and perioral musculature were stimulated by paired electrodes. The stimulation was at first applied every day, then every second day, and finally, to stabilize the effect achieved, once a week.

The results of the therapy were recorded by standardized clinical examination and photographically. They were evaluated by the modified »North Western University Disability Scale« questionnaire (2) revised by us for purpose of this examination. The elements scored by the questionnaire were walking (A), dressing (B), eating (C), and talking (D). Three instructive cases will be presented in more details.

Case 1: D. E., 50 years old, housewife. Last four years she had developed rigidity, first in the left and then in the right extremities. In the period preceeding her coming to the hospital she could no longer go out, or dress herself, or use the spoon or fork, or bring the cup to her mouth, or turn in bed.

The neurological findings on admission: The patient was able to walk and sit only if helped by somebody. When sitting, the defect of postural fixation was present, with the trunk falling back. When standing she could not turn to the left at all. She could turn to the right with much help only. She showed a marked progravitation posture,

propulsion was pronounced, and she was dragging her feet while walking. No tremor was observed. The fingers were considerably deformed due to contracture; the most severely attacked was the ulnar abduction in the metacarpophalangeal joints.

The patient has never before been treated for her disease. A favorable effect of stimulation was observed already after a week's therapy. She became far more independent in her walking, dressing, and eating. The improvement recorded after a month was assessed as a very significant one. She was moving round the ward quite on her own, she was dressing herself and was using the spoon without anybody's help. Asynergy at sitting entirely disappeared. The muscular rigidity was clearly reduced. The contracture, i. e. the ulnar deviation of the metacarpophalangeal joints, was considerably less pronounced.

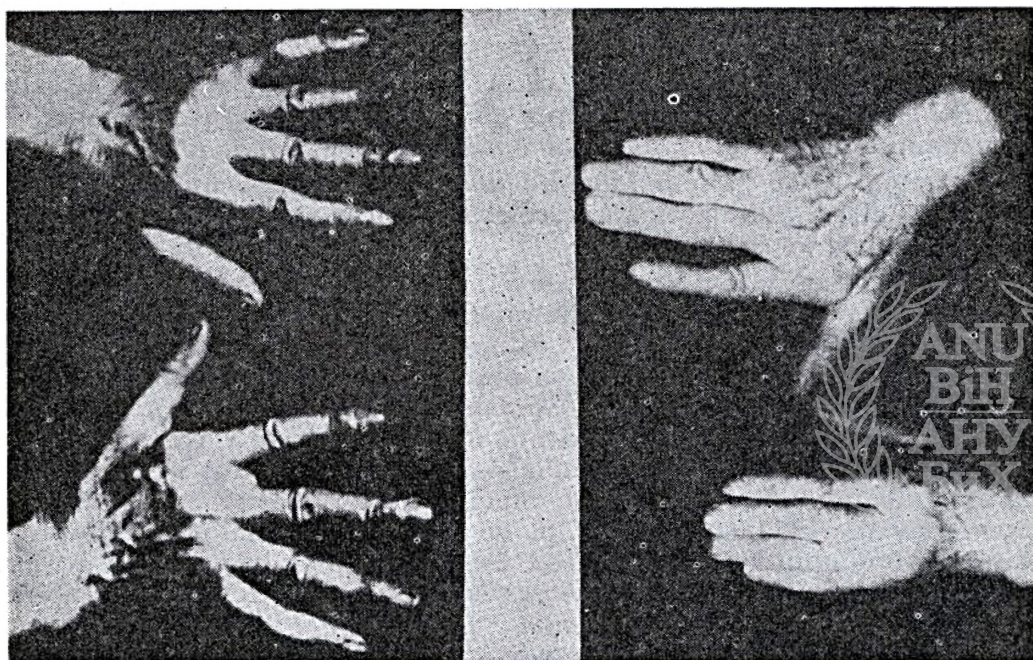


Fig. 1

The maximal finger abduction before and after a month of treatment.

Case 2: S. K., 73 years old male patient. He has developed Parkinson's syndrome, more markedly in the left extremities, in the last 5 years. His speech had been hardly intelligible in the last 2 years. Before the beginning of the therapy the patient could partly dress himself only by somebody's help. He was eating with difficulty and could not turn in bed easily. He was going out only if accompanied by somebody else.

The neurological findings on admission: A pronounced progravitation posture, strong propulsion, lack of associated movements, walking »à petit pas«. A markedly prolonged beginning of movements. Rigidity was more severe in the left than in the right side. Swallowing was impaired. Speech was slow and hardly modulated. He was not able to pronounce whole sentences but only individual words.

After 24 stimulations the therapy was interrupted. The patient was able to dress without anybody's help and to walk 3—4 kilometres. His speech became far more intelligible: practically, every word was clearly pronounced. Along with receded difficulties in swallowing, pseudohyper-salivation disappeared too.

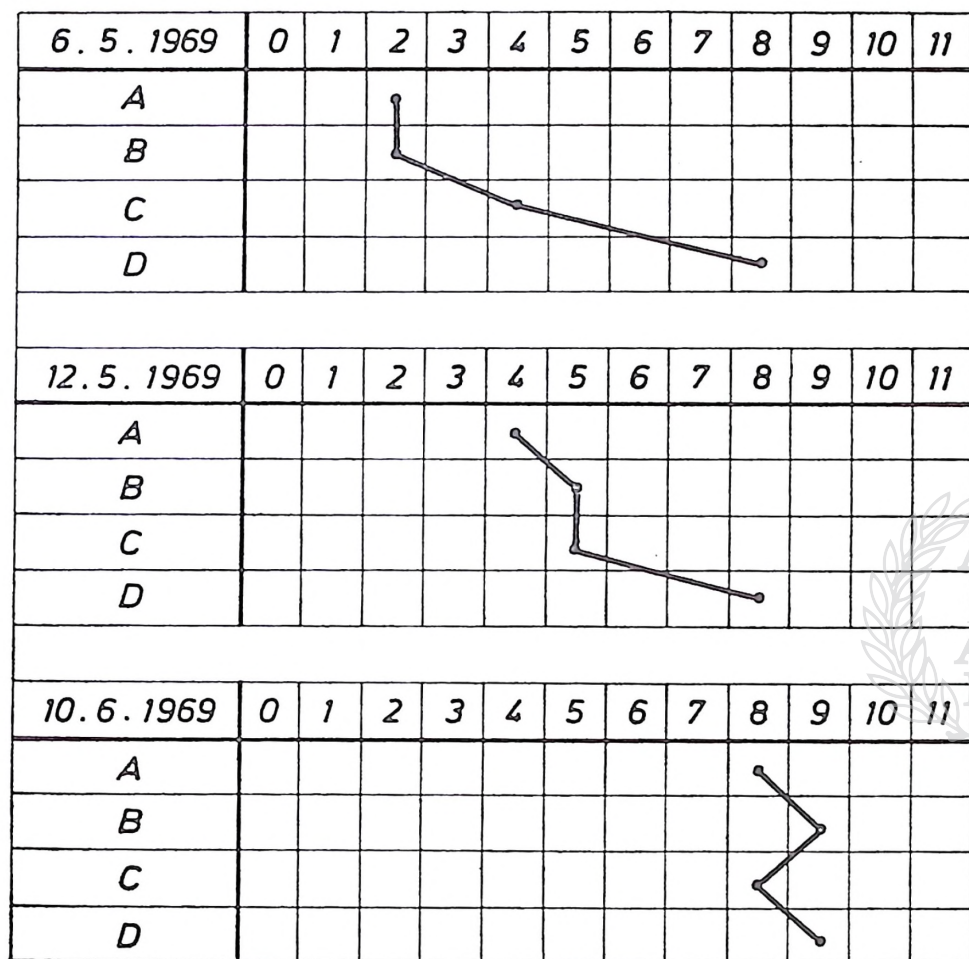


Fig. 2

Case 3: P. T., 50 years old, male patient. A bilateral stereotactic operation was performed on the patient. His principal disorder was anarthria with impaired swallowing and pseudohypersalivation. In addition to the standard stimulation scheme, the laryngeal region and the region of the low facial musculature were stimulated as well. After the first stimulation the patient was already able to speak clearly with-out change in speech modulation and rhythm. He was able to

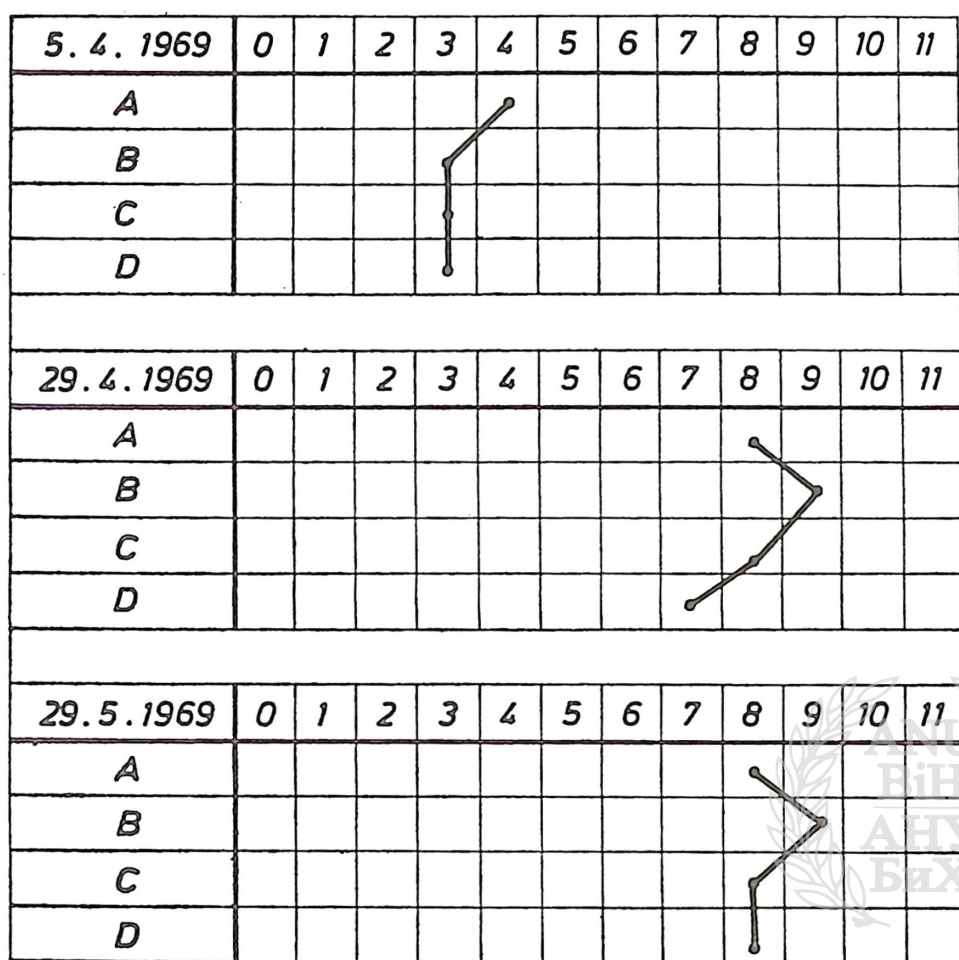


Fig. 3

pronounce not only individual words but also whole sentences. The effect lasted for more than one hour. After the second stimulation the duration of the positive effect was considerably shorter. After the third stimulation the positive effect did not develop at all.

DISCUSSION

These three cases were presented to show possible effects of alternating low frequency electrical stimulation on symptoms of Parkinson's disease, without pretension to analyse the whole group of patients on this occasion. We would like only to mention that results varied and

that in some cases the effect was negligible. Rigidity and akinesias have often been reduced. There was almost no effect on tremor.

The theoretical basis of the therapy according to Hufschmidt (1) lies in »passing on information« to motor centres by repetitive electro-stimulation. Afferent impulses provoked by the direct stimulation of receptors have an advantage over voluntary efferent impulses which only secondarily stimulate receptors and quickly lead to fatigue. The author of the method maintains that what is essential in the method is the excitement of the Golgi receptors which transfers the impulses transmitted through I b fibres to spinal interneurons. This interneuron system integrates impulses and transmits them to higher or lower segments and also to the contralateral musculature. The sensitivity of the spinal interneuron system in Parkinson's syndrome is considerably increased (Hufschmidt, 1960³).

Any movement disturbance, either due to a central or peripheral cause, results in a limited inflow of afferent information which keeps the movement programming motor centre active. There are experimental data which show the importance of afferent information for motor function. Electrostimulation and the repetitive stimulation of the musculature reactivate the functionally impaired central organ for which afferent impulses are the basis of its functioning. This fact, however, puts a limit to this kind of therapy. The therapy is successful only if there is a functional collapse (break-down) of the centre. The organic defect remains. This is the rationale of the author of the method (1).

The positive effect of this therapy lasts, as a rule, longer than the »cure« itself. To prolong it, it is necessary to repeat stimulation from time to time. Repeated stimulation appears necessary because repetition is the basis of both the production and the maintenance of conditioned reflexes. Indeed, the production of negative conditioned reflexes might be the very factor responsible for the missing of any favourable effect on rigidity and akinesias at the beginning of stimulation. To which extent humoral changes accompany functional ones is a problem which may become the subject of very interesting studies.

A. JUŠIĆ, S. DOĞAN I M. FRONJEK

EFEKTI NISKO FREKVENTNE MUSKULATURNE ELEKTROSTIMULACIJE NA EKSTRAPIRAMIDALNE SIMPTOME

KRATAK SADRŽAJ

H. J. Hufschmidt uočava povlačenje spastičnosti nakon tretmana niskofrekventnim električnim stimulansima. Prema njegovom mišljenju, efekat na spastičnost je baziran na stimulaciji Golgijevog organa, I b aferentnih vlakana i spinalnih interneurona. Kroz spinalne interneurone impulsi dopijevaju do kontralateralne strane ili se penju ka supraspinalnim strukturama.

U ovoj metodi upotrijebljena je dvostruka električna stimulacija sa intervalom od 1—1,5 ms. Intenzitet stimulacije je takav da izaziva vidljivu kontrakciju mišića.

Mi upotrebljavamo ovaj metod kod pacijenata sa Parkinsonovim sindromom. U većini slučajeva ovakav tretman je doveo do redukcije rigiditeta i do smanjenja hipokinezije. Nekog bitnog dejstva na tremor nije bilo. U nekim slučajevima čak je tremor postajao i jači.

Autori su prezentirali nekoliko slučajeva da ilustruju efekte.

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DISCUSSION

Bernard: Was electrostimulation therapy applied to patients already treated with anti-parkinson drugs? Did classical treatment influence the results obtained by electrostimulation therapy?

Jušić: We did not stop the classical anti-parkinsonien therapy in the patient under the electrostimulation. The results were better in nontreated patients. The spetial attention should be paid to the third ptientt reported.

ANDRÉ BARBEAU

SYMPOSIUM TREMENS*

Having been assigned the difficult task of summarizing this very interesting Symposium I have chosen to avoid the pitfalls of thorough and systematic review, by elaborating on what is definitely my own biased outlook on the mechanism of tremor, and incorporating what I think I have learned during these last two days. All of us came to the historic City of Sarajevo, at the invitation of Professor. P. Stern, who has been concerned with these problems for many, many years. We came to learn and to talk, and to think about tremor. To all intents and purposes what is tremor? What do we know now and what must we study in more details in the years to come?

To the clinician, tremor is an alternate contraction of flexors and extensors around a more or less fixed joint. It is endowed with specific rates and amplitudes which can be described and measured, but which change under special conditions. These must also be described, analysed and catalogued. Not all tremor is of parkinsonian origin and the laboratory investigator must avoid the errors of many clinicians. It is not yet clear in my mind what the relationship is between the tremor induced in animals by oxotremorine and that seen in patients. Shivering is not the rest tremor of Parkinson's disease, nor is it the intention tremor of cerebellar disorders! The only experimental tremor I have seen in animals which in some ways resembles parkinsonian tremor, is that induced by Reserpine.

In the end, the rate and amplitude of tremor must be organized and controlled at the periphery. Its final manifestation is in the coordinated action of alpha and gamma cells in the spinal cord and as they innervate the primary muscle spindle endings. We heard of the importance of that mechanism during this meeting (*Schäfer*). If there is no doubt about the sensory input modulation at this level, it probably cannot influence the actual generation of tremor. Approaches to this general problem have been presented in studies of the blink reflex (*Gregorič*), of tendon jerks and of controlled afferent inflows (*Dimitrijević*)

* This unusual title is adopted because of the chosen topic but also to remind us of the state of tremulousness, common to the other well known entity with a similar name, in which the speaker found himself before delivering this summary of the meeting.

and in therapeutic attempts involving low frequency muscular electro-stimulation (*Jušić*).

The true tremorigenic center is then situated above the monosynaptic reflex arc. Many levels of the neuraxis must be considered in the search for the so-called »pacemaker«. During this meeting we heard of the results of sections of the spinal cord in curare-induced shivering (*Ten Bruggencate*). At an other level we were told of studies on the electrical activity of the cortex as it relates to tremor and as it is modified by parkinsonian drugs (*Tóth*) or adrenergic and cholinergic influences (*Bernard*). An overview of drug induced rigidity and tremor was presented by *Jurna*. This author reminded us of the very important physiological observation that rigidity is abolished by removal of the striatum and that tremor produced by oxotremorine is of the gamma type while that induced by Reserpine is of the alpha type.

The only areas of the brain where independent firing cells with an *intrinsic* rhythm have been found in monkeys or in human stereotaxic studies, have been the posterior part of the ventrolateral nucleus of the thalamus and some components of the underlying subthalamic area (*Struppler*). This area is the most important target for a successful surgical approach to rest, postural or intention tremor. These cells which we, somewhat facetiously, have called »pulsating«, receive important afferents from the cerebellum, the brain stem and the basal ganglia. The possible role of serotonin components in the brain stem output of the rubro-olivo-dentato-rubral loop of Poirier have been emphasized by us. We feel that damage to this possible serotonergic pathway may result in hyperreactivity of the so-called »pulsating« cells. In this respect sleep studies carried out during L-DOPA treatment (*Kendel*) are particularly instructive, since tremor at night only appears during R. E. M. or unsynchronized sleep. This recalls the important investigations of Jouvett and his school on the role of serotonin in this system.

The other main input to the »pulsating« cells is from the basal ganglia. These nuclei, whose many pathways were thoroughly reviewed by *Gluhbegović*, may even be in some sort of an anatomical relationship to exterior configurations of the skull (*Hadžiselimović*). At any rate they play mainly the role of a filter in sensory-motor integration. In this basic function, dopamine plays an important role.

Thus introduced, the relationships between the many striatal putative neurotransmitters which Otto Loewi studied so long ago (*Naratil*), were thoroughly reviewed during this Symposium. *Potkonjak* reported experiments in which she relates brain levels of acetylcholine (ACh) and clinical rigidity induced by fluphenazine. There is now almost total agreement that tremor can be correlated with the levels of brain acetylcholine (*Oelszner*), and more accurately with ACh release (whose mechanism was studied in detail by *Vizi*) or the state of stimulation and saturation of muscarinic ACh receptors. The emphasis obviously now has to be placed at the level of the *receptor*, the neglected component of this system. The mechanisms of sensitivity and/or of tolerance of these receptors to various drugs must be investigated, as was well done after oxotremorine by *Huković* and by *György*. The elegant model

proposed by Župančić for the membrane-bound acetylcholinesterase of the cholinergic receptor should be the basis for many more important kinetic studies.

We would like to propose, as an area of potential reward, that the so-called »pulsating« cells of the thalamus and subthalamus be separated by the specific single cell isolation techniques (described in this Symposium by Pavlin) and that the molecular pharmacology of their many receptors be studied along the lines proposed by Župančić. We would suspect that it will be found that the same cell can have specific receptors for many transmitters and that, somehow, the interaction between the membrane activities of these receptors will account for the rhythmic intrinsic properties of these cells. Only when we visualize the molecular basis of this activity will we truly understand the mechanism of tremor and will we be able to rationalize therapeutic attempts.

Another important aspect of the present Symposium has been the emphasis given to the role of trace metals in the production of various forms of tremor. This is understandable, given the long-standing interest in this subject of our host. Professor Štern reviewed in detail the experimental models of extrapyramidal diseases, particularly the role of iron and of copper and their mechanism of action at cellular and enzymatic levels. We personally have stressed the fact that it is more likely that these substances act through slow modifications (deficiency or loading) and that their effect on symptoms is through reaction of underlying amine imbalances, particularly in the striatum. Trace metals have again been studied in various extrapyramidal entities, confirming the iron and copper changes previously reported in Wilson's disease and Huntington's Chorea and adding important new observations on possible abnormalities of aluminium in these illnesses (Bokonjić). We reported how iron or chronic manganese loading, or a chronic magnesium deficiency, could modify the striatal dopamine content, and Stanković described how manganese treatment affects cholinesterase activity in total brain and basal ganglia of rats.

If such studies on trace metals and electrolytes offer possibilities towards the eventual understanding of the pathogenesis of tremor, we cannot say that the present has been forgotten because of that emphasis. We were gratified to hear of the results of therapeutic trials with Amantadine (Schnieden) and with L-DOPA (Zec), although in both cases the effect on tremor was not as marked as that on other symptoms.

At the end of these long but fruitful sessions we should perhaps remind ourselves of some simple clinical and physiological truths: *tremor is only a symptom, it is not a disease*: If we have yesterday and today placed considerable importance on this manifestation, it should not obscure the fact that there may be many causes of tremor. It is even possible to change tremor into chorea or dystonia, or chorea into tremor, simply by modifying the underlying precarious balance between brain neurotransmitters, as every clinician who has used L-DOPA in Parkinson's disease or Fluphenazine in Huntington's Chorea could tell you.

Thus if today we understand better the symptom *TREMOR* and its physiology, we have not moved any closer to visualizing the etiology

of any diseases where tremor is present. Perhaps the investigation of trace metals may, in this way, be more important on the long run. Again our host would have manifested his scientific intuition in choosing this subject of study many years before most of us.

It is impossible to close such a Symposium without thanking, from the bottom of my heart, and with the certainty that the feeling is shared by all, our humble and so sympathetic Professor P. Štern. To him, to his ever smiling staff, to the Academy of Science and Art of Bosnia and Herzegovina, go our thanks for this most interesting Symposium on Experimental Tremor.

A. BARBEAU

SIMPOZIUM TREMENS

KRATAK SADRŽAJ

Sumirajući i spominjući sve autore — učesnike Simpozijuma, Barbeau je zahvalio Akademiji nauka i umjetnosti BiH i prof. Šternu za organizaciju Simpozijuma o eksperimentalnom tremoru. Postoje razni tremori, nije svaki tremor parkinsonske prirode, postoje mnogi uzroci tremora. Moguće je mijenjati tremor u horeu ili distoniju, ili obratno. Finalna manifestacija tremora je koordinirano djelovanje alfa i gama-stanica. Tremor izazvan oksotremorinom je gama-tipa, a onaj izazvan reserpinom je alfa-tipa. Jedini eksperimentalni tremor koji je on vidio kod životinja i koji sliči parkinsonskom tremoru je onaj izazvan reserpinom.

Istinski tremogeni centar, »pacemaker« ili pulzirajuće stanice locirani su u posteriornom dijelu ventro-lateralnog nukleusa. Oštećenje serotoninškog puta prema pulzirajućim stanicama može rezultirati hiperaktivitetom pulzirajućih stanica. Acetilholin u mozgu ili, bolje reći, oslobađanje acetilholina, njegovo vezivanje na receptore, kinetičke studije odnosa acetilholin — receptori na pulzirajućim stanicama — mogli bi dovesti do razumijevanja mehanizma tremora i konzekutivno tome do racionalnije terapije.

Tragovi metala koji mogu izazvati neravnotežu metabolizma amina u mozgu, mangan, aluminijum, bakar i željezo, ostali elektroliti, helati i metali i njihovo proučavanje — sve to može dovesti do eventualnog razumijevanja patogeneze tremora. Na koncu, Barbeau tvrdi da je tremor samo simptom a ne bolest, da danas bolje razumijemo tremor, njegovu patofiziologiju i etiologiju i da terapija amantadinom i L-DOPOM u nekim slučajevima ohrabruje.

Papers which have not been
presented



G. M. EVERETT

CHOLINERGIC AND BIOGENIC AMINE SYSTEMS IN THE ORGANIZATION OF MOTOR ACTIVITY

It is with deep regret that I cannot attend the symposium on Experimental Tremor and we wish it all success.

Our interest in this field goes back to our discovery of Tremorine in 1955. Since that time the number of papers in which this drug and its metabolite oxotremorine have been used as tools in Neuropharmacology has grown to a literature of hundreds of studies. We among others have long proposed the idea that the normal function of the motor system is dependent on a balance of cholinergic and biogenic amine systems. Tremorine and oxotremorine imbalance these systems by causing marked increases in acetylcholine (Holsted) and by causing decreases in either norepinephrine or dopamine (Everett, Friedman). Further, Gessa et al have shown oxotremorine causes a compensatory increase in dopamine turnover in the caudate. In our most recent studies we have shown that the tremor, rigidity and decreases in motor activity produced by oxotremorine (0.5 mg/kg i. p.) are antagonized by L-DOPA in doses of 200 to 400 mg/kg i. p. Likewise methamphetamine also antagonizes these motor deficits as does apomorphine. These findings support the thesis of rebalancing neuro-modulator system by increasing the levels of dopamine directly with L-DOPA or by giving dopaminemimetic drugs.

Thus tremorine and oxotremorine remain useful tools in preparing animal models on which to experiment and search for still more effective drugs against the various motor disorders in man.

Of equal importance in this field has been the use of rauwolfia alkaloids, which deplete the biogenic amines, resulting in a cholinergic dominant system and again producing a neuromuscular state resembling parkinsonism in animals and man. Our studies on the reversal of these abnormalities in mice and especially in monkeys by giving a MAOI and dl-DOPA (1957) and more recently with L-DOPA alone again supports the thesis of balanced systems involved in normal motor functions. Recently we have prepared the »Ultimate Parkinsonian Mouse«, or »Double Parkinsonian Mouse« if you prefer. The mice are first given a dose of 100 mg/kg deserpidine i. p. and four hours later, when full symptoms are present, a dose of oxotremorine (0.05 mg/kg i. p.) is given. These mice show decreased motor activity, tremor, abnormal posture

and ptosis. In these mice atropine only blocks tremor but not the other signs. However, L-DOPA is highly effective as its methamphetamine in reversing or decreasing these symptoms. Apomorphine (in high doses of 25 and 50 mg/kg i. p.) is surprisingly ineffective in this preparation. This model is also worthy of further study.

We should also point out the many unexplored compounds which will produce sustained tremor without signs of cholinergic stimulation. These remain for future experimentation. Finally, we should consider the good fortune we have had to find drugs which affect some of the basic biochemical modulators of the brain and thus offer us much closer models of neurological diseases than one might rightly have hoped for.

G. M. EVERETT

HOLINERGIČNI I BIOGENI AMINI U POSTANKU MOTORNE AKTIVNOSTI

KRATAK SADRŽAJ

Autor je 1955. godine otkrio tremorin. To je dalo povoda za nastanak jednog ogromnog broja radova i omogućilo je bolji studij statičkog tremora parkinsonskog tipa. Tremorin i oxotremorin uzrokuju porast acetilholina i pad dopamina i norepinefrina u mozgu. Kompenzatorno dolazi do porasta metabolizma dopamina u caudatusu. Sve se to može antagonizirati pomoću L-DOPE. To upućuje na zaključak da je tremorin idealna supstanca za izazivanje Parkinsonove bolesti na životinjama. Autor upozorava i važnost Rauwolfija-alkaloida za ovu vrstu ispitivanja, kao i inhibitora mono-amino-oksidade, kao i na uticaj amfetamina na inhibiciju raznih simptoma uzrokovanih tremorinom ili oxotremorinom.

F. W. KLUSSMANN, F. K. PIERAU, AND G. SPAAN

FREQUENCY AND SYNCHRONIZATION OF SPINAL α -MOTONEURONS DURING SHIVERING

The electromyogram of shivering animals can exhibit grouped discharges as a typical sign of such a cold tremor. Rhythm and frequency of these grouped voltages may vary within certain limits, for instance with intensity of shivering or with respiration. Yet the mean frequency of cold shivering of an animal species is dependent on its body weight, as was shown by Spaan and Klussmann (1970). Figure 1 was con-

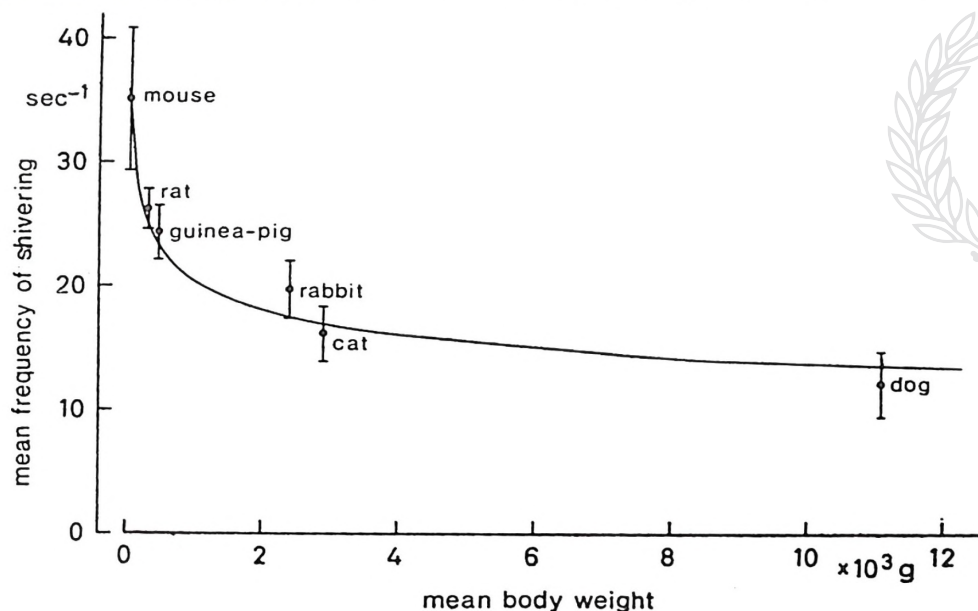


Fig. 1

Correlation between mean frequency of shivering of an animal species (ordinate) and its mean body weight (abscissa). Mean frequencies with standard deviations.

structed from the data of their experiments. It is obvious from this figure that the shivering frequency increases with decreasing body size, the frequency of the mouse (35/sec) being 3 times greater than that

of the dog (12/sec). The mean frequencies can be fitted fairly well by the drawn curve which was constructed from the equation $y = 70,73 \cdot x^{0,18}$ (Spaan and Klussmann, 1970), where y = mean shivering frequency (grouped discharges/sec) and x = mean body weight (g).

One of the open questions in this context is how the various motoneurons get synchronized to grouped discharges. And, furthermore, it is not clear if a single α -motoneuron discharges only once during each discharge group or several times, i. e., in bursts.

To answer these questions electromyographic and ventral root recordings were done in cats and dogs with the purpose to analyse the interspike intervals and to measure the degree of synchronization of different α -motoneurons and compare it with those of muscle spindle afferent discharges. A preliminary report of a part of the results has already been published (KLUSSMANN et al., 1969).

METHODS

Experiments were done on anesthetized (Nembutal, 30—40 mg/kg) or decerebrated cats and dogs. Shivering was induced by either peripheral cooling of the skin or central cooling of the spinal cord (SIMON et al., 1964). Electromyographic recording was done with concentric needle electrodes (Disa) from the hindquarters of the animals. After laminectomy activity of spinal motoneurons was recorded from filaments of ventral roots $L_6 - S_2$ with small platinum wire electrodes. For comparative purpose muscle spindle activity was recorded from dorsal roots $L_6 - S_2$. Amplification and registration was achieved with conventional electrophysiological electronic equipment.

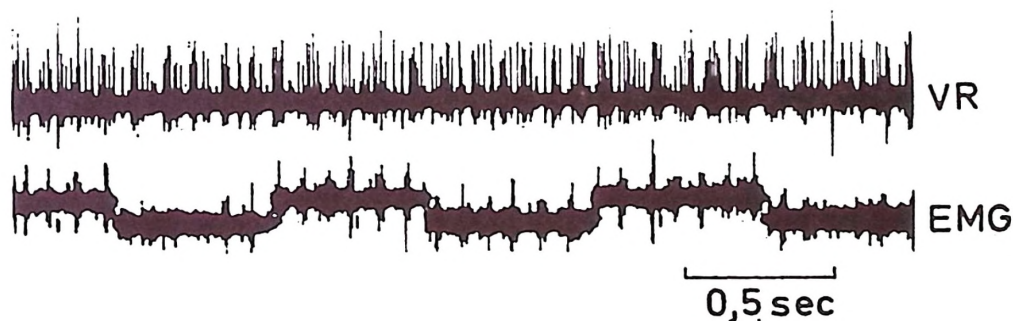


Fig. 2

Simultaneous recordings of motoneuron discharges from ventral root L_7 (VR) and of electromyographic activity (EMG) from hindleg of a shivering dog. Shivering was induced by cooling the spinal cord. Baseline of EMG shifted with respiratory rate, inspiration upwards. Spikes in this and the following figures have been slightly retouched for photographic purposes.

RESULTS

Figure 2 shows in the lower tracing the electromyogram from a cold shivering dog. Shivering was induced by isolated cooling of the spinal cord. The electromyogram reveals the typical grouped discharges. The change in baseline of the EMG-record was done in order to control variations in the rhythm of grouped discharges with respiration. The upper trace of fig. 2 shows the simultaneously recorded activity from a relatively thick filament of ventral root L_7 . The same grouping of motoneuron activity is evident in the ventral root recording, the frequency being in the same range as in the electromyogram. From many similar recordings of this kind it must be concluded that the grouping of electromyographic activity is caused already by a grouping of the motoneurons and cannot be generated within the muscle.

The recording from such a thick ventral root filament as in fig. 2, however, makes it impossible to determine the discharge rate of any single motoneuron. The question if any one motoneuron discharges only once or several times during any grouping can, therefore, only be answered by single fibre recordings, of which fig. 3 shows 2 examples. It is obvious that the discharge rhythm of the motoneuron in recording A is very regular except for a slight increase in frequency with the increase in shivering indicated by the increased electromyographic activity in the lower trace of record A. Part B of fig. 3 demonstrates the onset of firing of a single α -motoneuron with the beginning of shivering. In this case, too, discharge frequency became very stable soon after shivering had started.

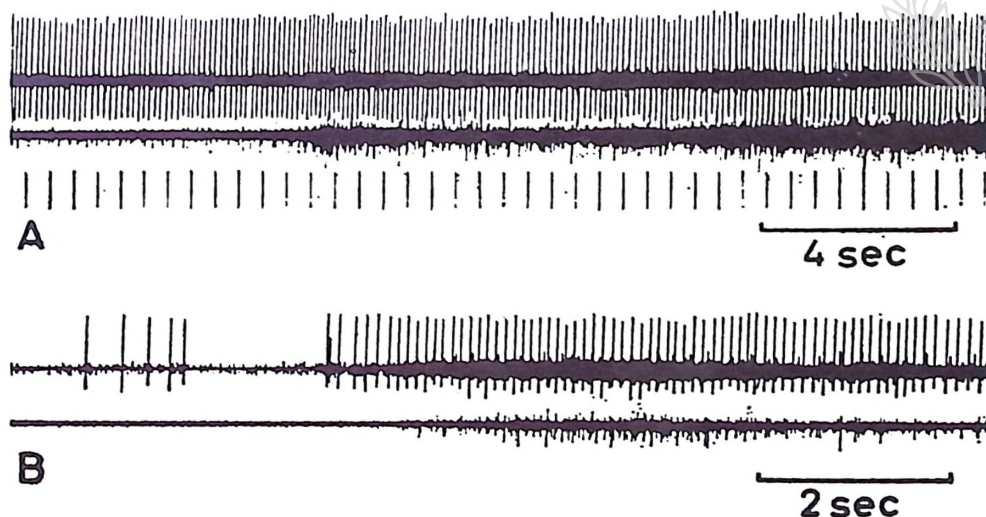


Fig. 3

Simultaneous recordings of single α -motoneuron activity from ventral root L_7 (upper tracings) and electromyographic activity from hindleg (lower tracings) in two different shivering cats (A and B). Shivering induced by cooling the spinal cord. Vertical bars in record A are 0,5 sec time marks.

From these recordings it must be concluded that the typical grouping of motor units during shivering cannot be caused by burst-like discharges of single motoneurons but must be caused by the synchronization of various α -motoneurons. The origin of this synchronization cannot lie in supraspinal centers since experiments with isolated cooling of the spinal cord have shown that shivering can also be induced in spinalised animals (Simon et al., 1966; Klusmann et al., 1966; Kosaka and Simon, 1968 a, b). In addition Birzis and Hemingway (1957) were unable to find any grouped discharges in their recordings from the so-called »shivering pathway« in the medulla. Only two possibilities, therefore, remain how the grouped discharges might be generated: 1) The afferent impulses from muscle spindles and other receptors, which form the driving input for the α -motoneurons are already grouped. 2) The synchronization occurs at the spinal level by an intraspinal mechanism.

To decide between these two alternatives a greater number of ventral and dorsal root recordings were analysed in the following manner (fig. 4). Each interval between two consecutive impulses of a

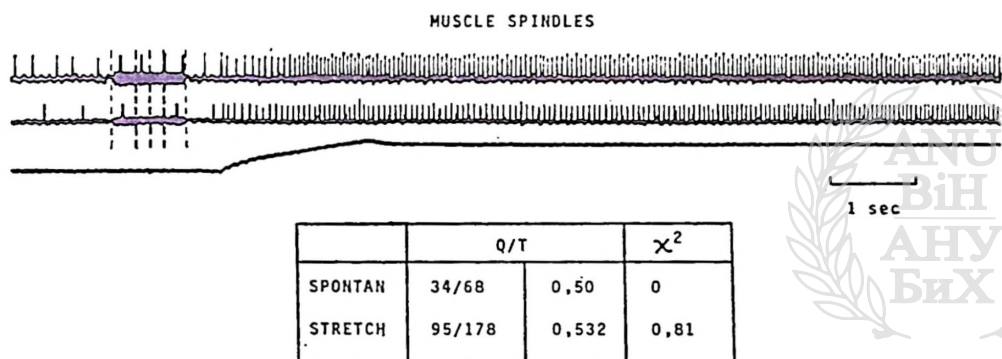


Fig. 4

Spontaneous activity and stretch responses of two simultaneously recorded muscle spindle afferents (conduction velocities: 55 m/sec upper recording; 75 m/sec lower recording) to stretch of m. gastrocnemius (3. trace). The shaded area indicates how one of the interspike intervals of the lower recording was divided into four equal parts. In this case 3 of the four spikes of the upper recording fell »close« to the two spikes of the lower recording. The numbers in the inset give the number (Q) of those spikes of the upper recording which fell »close« to a spike of the lower recording in proportion to the total number of spikes in the upper recording (T). For definition of »close« see text.

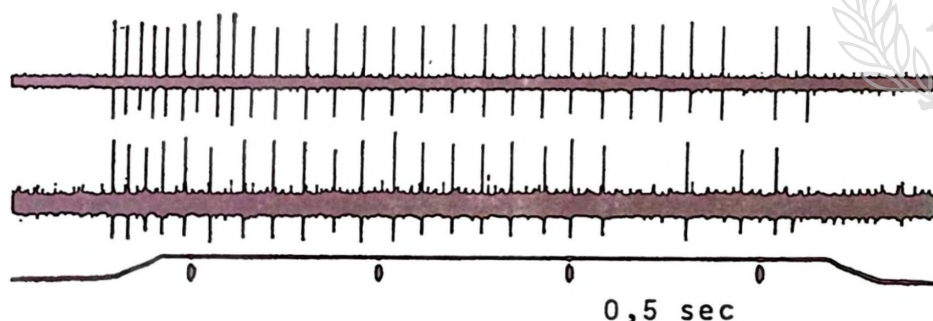
muscle spindle or an α -motoneuron recording was divided into four parts of equal length. The impulses of a second simultaneously recorded muscle spindle or an α -motoneuron was then tested if they fell »close« to the impulses of the first recording. The impulses of the two recordings were considered to be »close«, if the second impulse fell into the two interval-quarters adjacent to the first spike. The principle of this

measurement is shown in fig. 4. In each recording the number of »close« impulses was counted separately for spontaneous activity and for responses to muscle stretch.

This number of »close« spikes (Q) was set in proportion to the total number of spikes so tested (T). If the impulses in the two recordings occur independently from each other the impulses of the second recording should be equally distributed over the four interval-quarters of the first recording, i. e. the number of »close« spikes (Q) should be about half of the total number (T). If, on the other hand, synchronization plays a role, the number of the »close« spikes should be more than half of the total number. The null-hypothesis that the impulses of two recordings occur independently and have a stochastic distribution was tested with the chi-square method.

As can be seen from fig. 4 during spontaneous activity of two muscle spindles, of which only a small part is shown, a total of 68 impulses of the upper recording was tested for »closeness« with spikes in the lower recording. 34 of the 68 impulses, i. e. 50%, were found »close« to a spike in the lower recording. During stretch this proportion of »close« spikes is very similar as can be seen from the inset of fig. 4. From 14 different experiments with two muscle spindle recordings in each, a total of 1463 impulses was tested during spontaneous activity. 746 of these 1463 impulses, i. e. 51%, fell »close« to a spike of a second recording. During muscle stretch a total of 2818 spikes was measured

α -MOTONEURONS



	Q/T		χ^2
SPONTAN	-	-	-
STRETCH	19/22	0,863	11,64

Fig. 5

Stretch responses of two α -motoneurons (upper and middle trace) to stretch of *ns. gastrocnemius* (lower trace). For meaning of inset see fig. 4.

of which again about 51% fell »close« to the other impulses. Applying the chi-square test to these numbers the null-hypothesis, that in any pair of spindle receptors so tested the two receptors discharge independently of each other, has to be accepted.

A different picture, though, evolves if the discharges of two simultaneously recorded α -motoneurons are tested in the same way. An example is given in fig. 5. In this case no spontaneous activity was present in these two filaments. During stretch 19 of 22 impulses (see inset of fig. 5) of the upper recording, i. e. 86%, fell »close« to a spike of the lower recording. The chi-square test in this example was highly positive ($0,0005 < P < 0,001$). In 11 experiments with recording from two ventral root filaments a total of 1597 α -motoneuron impulses were counted during spontaneous activity and 2364 impulses during stretch responses. During spontaneous activity 898 impulses out of 1597 fell »close« to a spike in the second recording, i. e. 56%. This percentage increased to 61% during stretch. Tested by the chi-square method (table 1) such a high number of synchronized spikes cannot be accounted for by randomness. The statistical significance of this synchronization is very high as is evident from table 1.

The over-all percentage of 61% of synchronized spikes may at first seem to be relatively small. The total count of impulses, though, included a great number of recordings in which no synchronization was evident at all. The reasons for these α -motoneurons to discharge asynchronously could be that they are lying too far apart in the spinal cord, or that they belong to functionally different motor units. It could also well be that for proper functioning they need to discharge in an asynchronous manner (see discussion).

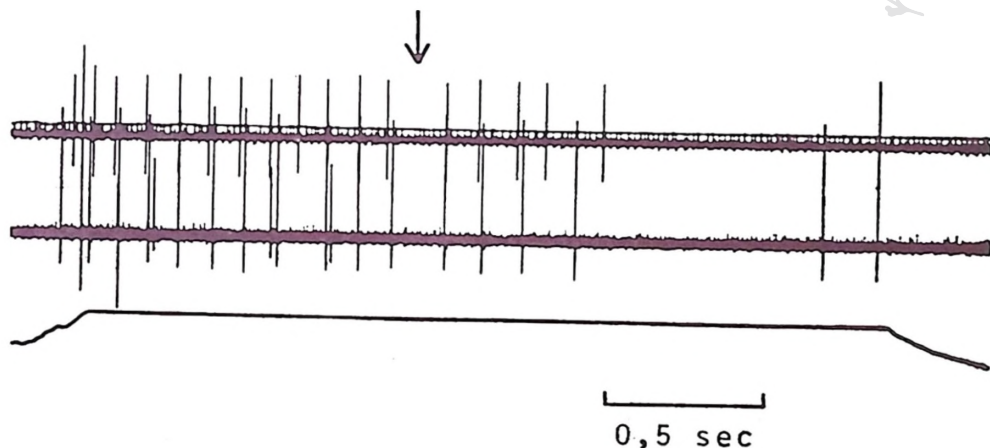


Fig. 6

Simultaneous recordings of stretch responses of two α -motoneurons to stretch of m. gastrocnemius (lower trace). Arrow indicates simultaneous change of units of both upper and lower in frequency recordings (see text).

In contrast to these asynchronous impulses, however, many other recordings revealed a high degree of synchronization of almost 100%. Fig. 6 gives an example. It is obvious that the unit of the upper recording is synchronized with the bigger unit of the lower recording in almost all cases. This is even true when a change in the regular frequency of the upper unit occurs (arrow). A third unit, too, which discharges a few times in the lower recording is highly synchronized with the other two.

DISCUSSION

The experiments had revealed that the activity of two simultaneously recorded muscle spindle afferents apparently occur independently of each other, i. e. they are not synchronized with any statistical significance. The impulses of two α -motoneurons, on the other hand, recorded in the same way did show, on the average, a statistical significant degree of synchronization. In a number of cases, this synchronization was almost 100%. The conclusion, which has to be drawn from these experiments, therefore, seems to be that the synchronization of α -motoneurons occurs at the spinal level and not through a synchronized input from muscle spindles. This is in agreement with the findings of Meurer et al. (1965), who were able to produce shivering with typical grouped discharges by local spinal cooling in dogs, which were deafferented previously. Stuart et al. (1966), too, have found grouped discharges in ventral root recordings in cats which were cooled and curarized. A synchronized afferent input which might arise from the rhythmically contracting muscles during shivering was absent in these experiments.

The experiments had also shown that each motoneuron discharged only once during each grouping. This is in agreement with the findings of Taylor (1962) who also found in electromyographic recordings during voluntary contraction that each motor unit fired only once during any grouped voltage.

The conclusion from our experiments, however, differs from those which have been drawn by Taylor (1962). According to Taylor the grouping of action potentials during slight voluntary contractions of peripheral muscles and diaphragm might be a purely chance appearance caused by the fact that the various motor units discharged at the same rate. The results of the experiments as described in this paper, however, clearly indicate, that synchronization of α -motoneurons does take place

in a great number of recordings. The neuronal mechanism, by which this occurs, remains unknown so far. One reason for the different findings of Taylor (1962) and our experiments could be that for slight contractions especially for the diaphragm to function properly a higher degree of synchronization of the α -motoneurons need to be avoided. During shivering, however, the grouping of motoneurons cannot be explained by the fact that the various α -motoneurons discharge at the same rate. If this were the case, the grouping of the muscle spindle discharges should have shown the same degree as those of the α -motoneurons since the discharge frequencies of any two spindle afferents were in many cases very much alike for the same muscle tension.

The degree of synchronization in our experiments increased with muscle stretch. This is not to surprising since the number of discharging α -motoneurons increases with muscle stretch. If there is a mechanism active at the spinal level which, for some reason, synchronizes α -motoneurons, this mechanism should become the more apparent the higher the degree of α -motoneuron activity. In addition, if it is considered that the phasic α -motoneurons have a higher threshold for reflex, supraspinal and other forms of excitation (Granit et al., 1957; Henneman et al., 1965a; Stelter and Klussmann, 1969) than the tonic α -motoneurons, it could well be that the degree of synchronization of α -motoneurons is coupled with the kind of α -motoneurons activated. Tonic α -motoneurons which mainly serve for posture, tonus and continuous isometric contractions would function properly only if they are synchronized as little as possible, because only then a maximum of smoothness of the muscular contraction is guaranteed. For fast contractions, however, with a great movement (isotonic contractions) a highly synchronized discharge of a great number of α -motoneurons is necessary by definition. Phasic motoneurons probably play a dominant role at the latter type of contractions and it could very well be that the synchronization, which was observed in our experiments, is especially important for the phasic α -motoneurons. These considerations may also explain why, in the beginning of cold shivering, almost no grouped discharges in the electromyogram and no tremor movements can be detected although the overall electromyographic activity has increased (Stuart et al., 1966; Göpfert and Stüfeler, 1952) as well as the oxygen consumption of the animal. With continuing cold load, however, cold tremor becomes more and more obvious as well from the visual aspects as from the increased incidence of synchronized discharges of motor units in the electromyogram. It could well be that the latter finding is correlated to the fact that more and more α -motoneurons of the phasic type get activated with increasing cold tremor.

Table 1

QUOTIENT OF THE NUMBER OF IMPULSES (Q) WHICH FELL »CLOSE« TO ANOTHER SPIKE OF A SECOND RECORDING TO THE TOTAL NUMBER OF SPIKES (T) TESTED; χ^2 — AND P-VALUE CALCULATED FOR THE HYPOTHESIS THAT $Q = T \cdot 0,5$

MUSCLE SPINDLES		α -MOTONEURONS			
	Q/T	χ^2	P	Q/T	χ^2 P
SPONTAN	746/1463	0,510	0,57	0,40 < P < 0,50	898/1597 0,563 24,80 P < 0,0005
STRETCH	1439/2818	0,511	1,28	0,20 < P < 0,30	1449/2364 0,612 120,62 P < 0,0005



SUMMARY

1) Ventral and dorsal root recordings were done in anesthetized cats and dogs during cold shivering induced by peripheral or spinal cord cooling.

2) Recordings from thick ventral root filaments revealed typical groupings of the α -motoneuron discharges in the rhythm of the cold tremor. From single fibre recordings it was concluded that each α -motoneuron discharges only once during any grouping and that grouped discharges originate from the synchronization of various motoneurons.

3) A statistical analysis (chi-square method) of the occurrence of synchronized impulses in a greater number of muscle spindle and α -motoneuron recordings revealed that α -motoneurons, are, in many cases, synchronized with statistical significance while the muscle spindle discharges occur independently of each other. From these experiments it is concluded that the α -motoneurons are synchronized at the spinal level and not through a synchronized input from muscle spindles.

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FREKVENCIJA I SINHRONIZACIJA SPINALNIH α -MOTO-NEURONA ZA VRIJEME DRHTANJA

KRATAK SADRŽAJ

1. Registrovani su električni impulsi sa ventralnih i dorzalnih korjenova kod anesteziranih mačaka i pasa za vrijeme drhtavice prouzrokovane klacenjem periferije ili medulle spinalis.

2. Registrovanje filamenota debelog ventralnog korijena je otkrio grupiranje tipično za električno pražnjenje α -motornog neurona u ritmu hladnog tremora. Registrovanje pojedinačnih fibrila je dovelo do zaključka da se svaki α -motorni neuron samo jednom električki prazni za vrijeme bilo kog grupiranja i da grupirano električno pražnjenje potiče od sinhronizacije različitih motornih neurona.

3. Statistička analiza (chi-square metoda) pojave sinhroniziranih impulsa u većem dijelu mišićnih vretena i registrovanja α -motornog neurona su otkrili da su ovi neuroni u mnogim slučajevima sinhronizirani, statistički signifikantno, dok se električno pražnjenje mišićnih vretena pojavljuje nezavisno jedno od drugog. Iz ovih eksperimenata je zaključeno da su α -motorni neuroni sinhronizirani na nivou medulle spinalis, a ne mišićnih vretena.

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N. ROSIĆ, B. BOŠKOVIĆ AND V. VOJVODIĆ

DEPRESSION OF ACTIVE AVOIDANCE BEHAVIOR IN RATS BY SMALL DOSES OF OXOTREMORINE

Chalmer and Erickson (2) have demonstrated that subtremerogenic doses of tremorine produced a blockade of avoidance responding, both in Warner shuttle-boxes and in Skinner-boxes (Sidman avoidance). This blockade of avoidance responses (AA responses) could be prevented by atropine, but not by methantheline, a quaternary anticholinergic drug. These experiments, as well as many others (7, 1), confirmed a cholinergic nature of avoidance behavior and supported the hypothesis given by Carlton (1), that cholinergic system in brain is involved in control of animal behavior.

As concerns cholinesterase reactivators the evidence available to date indicates that certain compounds can cross blood-brain barrier, and counteract at least in the part the central effects of some anticholinesterase inhibitors (3, 5, 6, 8).

Present paper deals with the effects of oxotremorine (OTR) on AA behavior in rats. It was assumed that OTR, as active metabolite of tremorine, would also produce a blockade of AA responses. The efforts were made to find out a minimal dose of OTR, which will provoke blockade of AA responses in well trained rats (Experiment 1). In experiment 2, the interaction between TMB-4, F-oxime and OTR were studied.

METHOD

Subjects. Adult male rats of Wistar-derived strain were used.

Apparatus. The apparatus consisted of series of automatically operated shuttle-boxes (commercial product of Ugo Basile — Milano). The source of conditioned signal (CS) was light mounted on the centre of ceiling. The stimulus lacked directional properties.

AA pretraining. The rats were pretrained in sessions, each consisting of 50 trials at 30-second intervals. The interval between sessions was 24 hours. The other conditions of test were: CS-US (unconditioned stimulus) = 7 seconds. CS precedes US for 3 seconds. US = 1.5 mA scrambled footshock. CS terminated by avoidance response; an escape response

terminated both, CS and US. If animal does not cross from one to another part of shuttle-box during the presentation of CS-US, both stimula terminated automatically. Such training was continued until the animals reached a stable performance with 40 or more AA responses per session.

Drug treatment. OTR in water solution was injected subcutaneously, while all other tested drugs were given intraperitoneal. Total volume of each injection was 0.1 ml/100 gr. of body weight.

In all experiments behavioral tests without treatment were carried out in the days before and after treatment sessions.

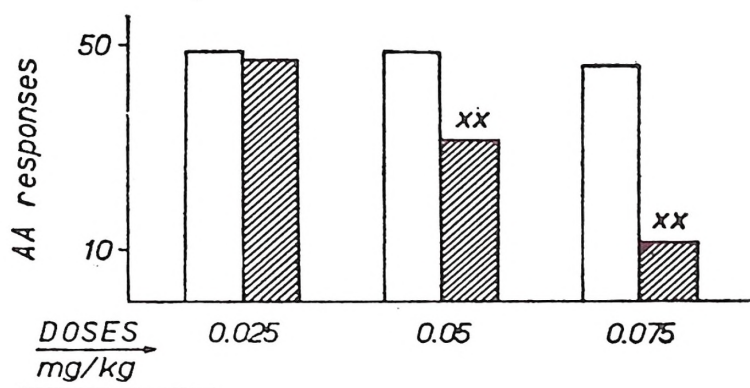
RESULTS

Experiment 1

In these series of experiments the dose response relationship and time course of OTR effects on AA behavior were investigated. All experiments were carried out with groups of well trained animals assigned at random to either dose of OTR, or to a time of testing after administration of drug. Doses of 0.025, 0.05 and 0.075 mg/kg of OTR were used, respectively. Fifteen minutes after single injection of OTR, all groups of rats were tested in shuttle-boxes. The results of these experiments are shown in Fig. 1.

Fig 1

EFFECTS OF VARIOUS DOSES OF OTR ON AA PERFORMANCE IN RATS



xx $P < 0.001$ ("t-test" for paired observation)

Fig. 1

Effects of various doses of OTR on AA performance in rats. All animals were tested 15 minutes after OTR administration. Columns refer to mean numebrs of AA responses given before OTR treatment (white columns) and after OTR injection (shaded columns).

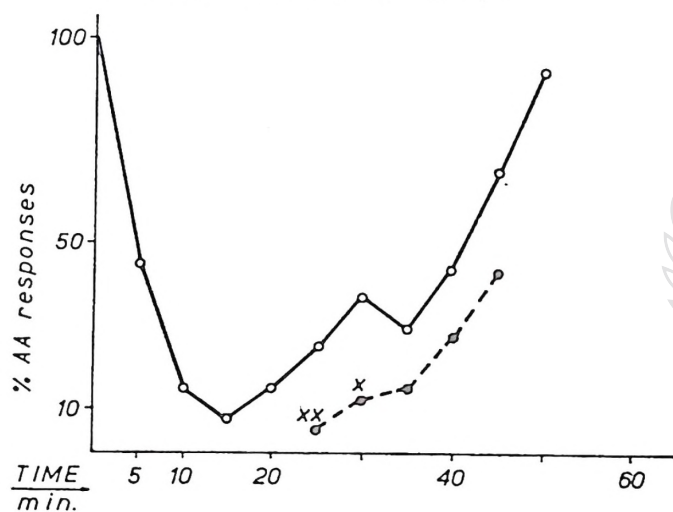
The subcutaneous injection of 0.05 and 0.075 mg/kg of OTR provoked marked depression of AA responses in rats, while the dose of 0.025 mg/kg failed to show any effect. The depressant effect on learned behaviour was shown, both by loss of AA responses and by return to an escape pattern, without motor incapacitation of treated rats. The symptoms of OTR intoxication (tremor, salivation, etc) were negligible.

The time course of OTR depressant effects was studied in rats treated with 0.075 mg/kg. Immediately after drug administration, animals were *continuously* tested in shuttle-boxes until a full recovery of AA responses were recorded.

As it is shown on Fig. 2, the depression of AA responses occurs 5 minutes after drug administration, reaching a maximum between 15 and 20 minutes. After this time, slow, but progressive recovery of

Fig 2

TIME COURSE OF AA DEPRESSION
INDUCED BY 0.075 mg/kg OTR



xx P 0.001 } "t-test" for independent
x P 0.05 } observations

Fig. 2

Time course of AA depression induced by OTR (0.075 mg/kg). The curves show average percentage of given AA responses in blocks of 5 minutes of performance. Upper curve=performance of rats which have been tested continuously immediately after OTR injection. Lower curve = performance of animals which have been tested 20 minutes after OTR injection.

AA responses was registrated. The number of AA responses reached the normal values about one hour of testing. In order to exclude the influence of relearning factor during the continous testing, a separate group of trained animals were injected with same dose of OTR and

tested 20 minutes later. In this group of rats a more marked depression was recorded (lower curve on Fig. 2) comparing with »continuously« tested group of animals. Mathematical analysis of blocks of AA responses (5 minutes performance per one block), has shown significant differences between means of AA responses in two experimental groups. However, this difference was significant only in initial phase of performance. This means that relearning factor could not be avoided in such design of experiments.

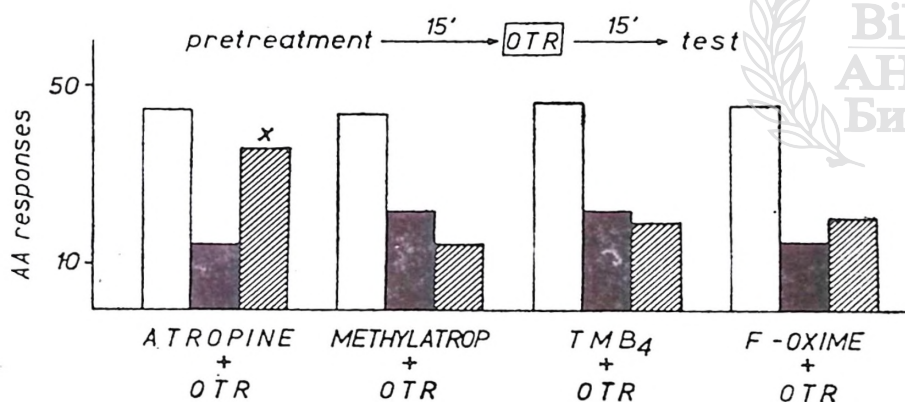
Experiment 2

Experiment 2 deals with the effects of anticholinergic drugs and cholinesterase reactivators on depression of AA behavior induced by 0.075 mg/kg of OTR. The following tested drugs were used: Atropine sulphate (5.0 mg/kg), atropine methyl nitrate (3 mg/kg), N, N'-trimethylenebis (pyridinium-4-aldoxime) dichloride (TMB-4) - 25 mg/kg and l-phenacyloxime-quinolinium chloride (F-oxime) in 12.5 mg/kg.

Experiments were carried out with groups of trained animals assigned at random to drug which has been used in experiments. Effects of tested drugs were studied with cross over experiments. All animals

Fig 3

EFFECTS OF ATROPINE, METHYLATROPINE, TMB₄ AND F- OXIME ON DEPRESSION OF AA INDUCED BY OTR



* $P < 0.001$ comparing with results of placebo + OTR experiments

Fig. 3

Effects of atropine, methylatropine, TMB-4 and F-oxime on depression of AA behavior induced by 0.075 mg/kg of OTR.

Cross-over experiments. Schedule of test: pretreatment — 15' — 15' — test. The columns refer to average numbers of AA responses given:
 — in control test (white columns)
 — after placebo + OTR (black columns)
 — after pretreatment with tested drugs (shaded columns).

in each group were tested twice. In the first experiment, one half of animals in each group were treated with placebo and OTR, and other half with one of tested drug and OTR. One week later, in second test, two conditions of drugs treatment were exchanged. The results of these experiments have been analyzed by means of »t-test« for paired observations, since each animal contributed scores to each of the two drug-treatment conditions. Animals were pretreated with placebo or with tested drugs 15 minutes before OTR administration, and 15 minutes later, they have been tested in shuttle-boxes. It has to be mentioned that all animals showing less than 30% loss of AA responses during placebo+OTR treatment were discarded from mathematical analysis, since the conditions of experiments required sufficient amount of AA depression induced by OTR alone.

Results of these experiments are shown in Fig. 3.

Results of experiments show that all animals treated with placebo + OTR exerted behavioral depression amounted to about 60% of the initial values.

When animals were pretreated with atropine, very low degree of AA depression was recorded. These animals performed AA behavior practically with the same rate as it was recorded in control tests.

In all other groups of pretreated animals the degree of AA depression was about the same as it was recorded when they have been treated with placebo + OTR injections.

DISCUSSION

As expected, OTR, like other cholinergic drugs, caused depression of AA behavior in rats. Atropine, but not methylatropine, blocked depressant effects of OTR. Such results indicated cholinergic nature of neurophysiological mechanisms underlying this pattern of AA behavior. From behavioral point of view, depressant effects of OTR are in accordance with Carlton's hypothesis (1), which postulated the role of cholinergic brain mechanisms in the control of animal behavior. Namely, stimulation of cholinergic system after administration of OTR, consequently reinforced those psychophysiological mechanisms which are responsible for inhibiting effects in animal behavior.

Absence of antagonism between oximes and OTR does not exclude the evidence of weak anticholinergic action of these drugs. There are many other factors which have to be investigated in order to detect anticholinergic actions of oximes in behavioral studies. Some of these factors are: lower sensitivity of AA behavior, necessity of large scale experimentation on dose levels and timing using different oximes, etc.

Finally, observed effects of OTR were obtained by using rather small dose range. It would be interesting, from pharmacological point of view, to consider a possible mechanism of above mentioned effects. Holmstedt (4) reported that doses of 0.5 mg/kg and higher, must be administered to rats in order to obtain evident rise of Ach in brain.

Accordingly, there is small probability that depressant effects of OTR on AA behavior, obtained in these experiments, could be ascribed to the action of OTR by mobilizing Ach.

CONCLUSIONS

The effects of subtreemorogenic doses (0.025, 0.05 and 0.075 mg/kg) of OTR on active avoidance behavior in rats were investigated. Except dose of 0.025 mg/kg, the other doses produced a marked depression of active avoidance behavior in rats. Depression occurred early after administration of OTR, reaching the maximum between 15 and 20 minutes. The effects disappeared 50 minutes after injection of OTR.

Depressant effects of OTR could be prevented by atropine, but not by methylatropine, TMB-4 and F-oxime, respectively.

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DEPRESIJA »PONAŠANJA IZBJEGAVANJA« KOD ŠTAKORA SA NISKIM DOZAMA OKSOTREMORINA

KRATAK SADRŽAJ

Ispitivani su kod štakora efekti supotreemorogenih doza (0.025, 0.05 i 0.075 mg/kg) OTR na aktivno »ponašanje izbjegavanja« (avoidance behavior).

Osim doze 0.025 mg/kg, druge doze su izazivale izrazitu depresiju aktivnog »ponašanja izbjegavanja« kod štakora.

Depresija se javljala ubrzo poslije davanja OTR, postizujući maksimum između 15 i 20 minuta. Efekti iščezavaju 50 minuta poslije injekcije OTR. Depresivni efekti OTR mogu biti spriječeni atropinom, ali ne metilatropinom, TMB-4, odnosno F-oximom.

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