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FREQUENCY AND SYNCHRONIZATION OF SPINAL α-MOTONEURONS DURING SHIVERING

The electromyogram of shivering animals can exhibit grouped discharges a as 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 wieght, as was shown by S p a a n and K l u s s m a n n (1970). Figure 1 was con-

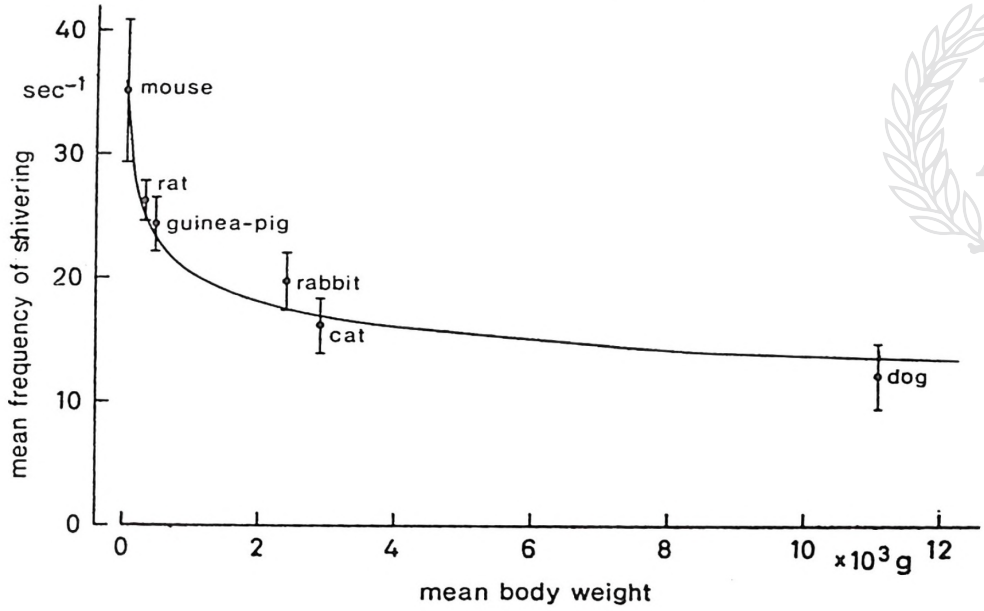


Fig. 1

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 $y=70{,}73 \cdot x^{0{,}18}$ (S p a a n and K l u s s m a n n, 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.

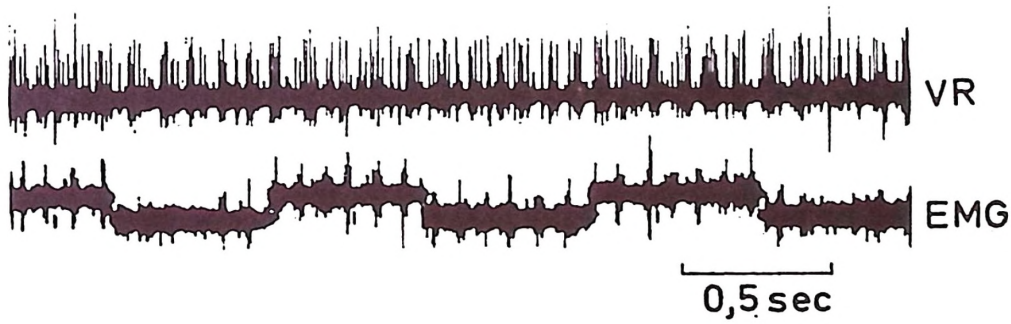


Fig. 2

Simultaneous recordings of motoneuron discharges from ventral root L_7 (VR) and of electromyographic activity (EMG) from hindleg of a shivering dog. Shivering was inuced 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.

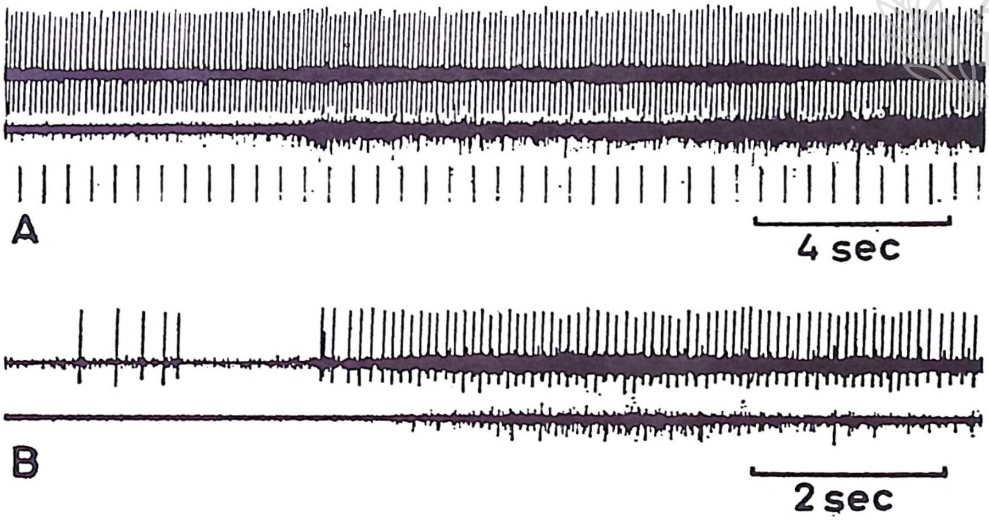
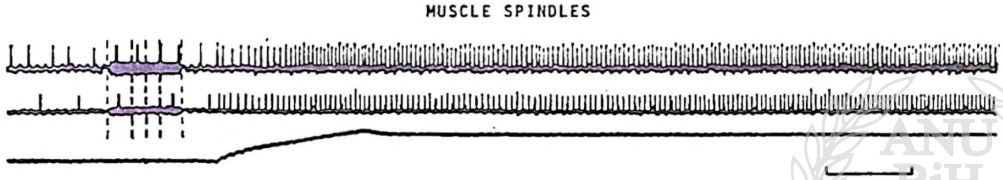


Fig. 3

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 (S i m o n et al., 1966; K l u s s m a n n et al., 1966: K o s a k a and S i m o n, 1968 a, b). In addition B i r z i s and H e m i n g w a y (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



	Q/T		χ^2
SPONTAN	34/68	0,50	0
STRETCH	95/178	0,532	0,81

Fig. 4

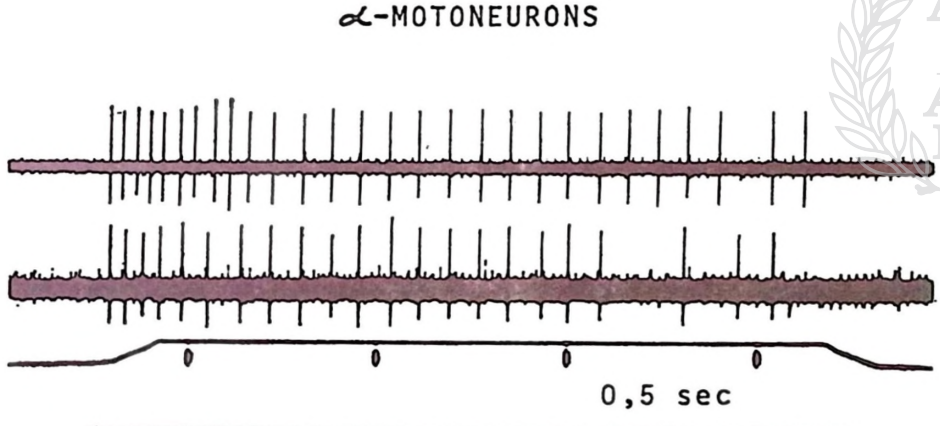
Spontaneous activity and stretch responses of two simultaneously recorded muscle spindle afferents (conduction velocities: 55 m/sec upper recording; 75 m/sec lower recording) to stretch of m. gastrocnemius (3. trace). The shaded area indicates how one of the interspike intervals of the lower recording 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



	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 m. 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 to 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).

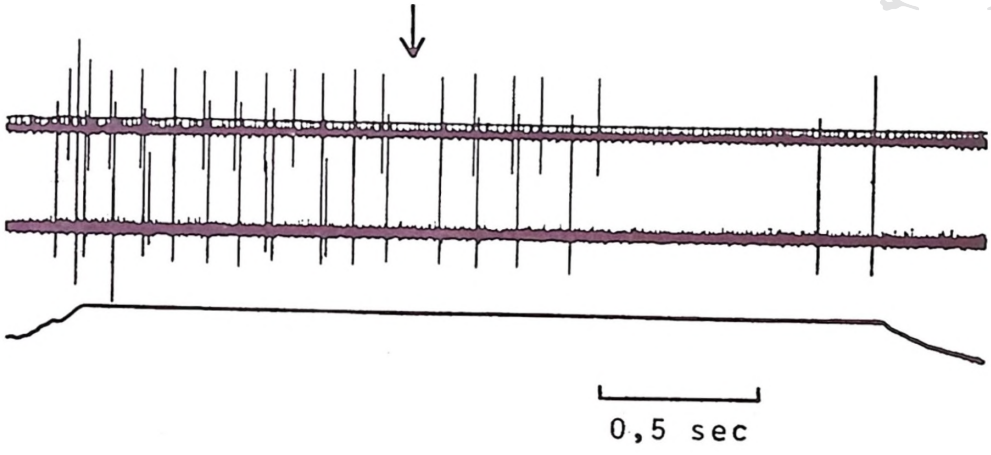


Fig. 6

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 Stufler, 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 αmotoneurons 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 klaćenjem 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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