Effect of Training on the Properties of Isolated Skeletal Muscles

In a recent investigation into the separate effects on intensity and duration of training routines (running on an endless belt) on the size of muscle fibres, it was demonstrated that, albino mice trained for 15 min/day at 0.83 ft/sec for 3 weeks, showed an increase in mean fibre cross-sectional area of 49.0% (WALKER[1]). The present report relates this change in fibre size to changes in the mechanical properties of the isolated muscles.

After training was complete the biceps brachii and the tibialis anterior were isolated. The right fore and hind limbs were amputated close to their respective limb girdles and thread was attached to the proximal tendon in the case of the biceps brachii and the distal tendon in the case of the tibialis anterior. These tendons were subsequently severed and the preparation pinned to a cork board through the bone left attached to the remaining tendon, and immersed in a constant temperature (35 °C) bath containing 150 ml mammalian Ringer. The solution was aerated and gently stirred by bubbling a mixture of oxygen (95%) and carbon dioxide (5%) through it. One platinum electrode was wound around the muscle and the other dipped into the Ringer's solution. Iso-metric measurements of muscle strength were recorded on a Physiograph (E and M Instrument Company). The loading tension in every case for both muscles was 3.8 g. The characteristics of maximal single twitches were measured following a single pulse of 50 volts lasting 2 msec. The muscle was put into tetanus using one hundred 50 V pulses/sec. These values were found to give the maximal response. The trained muscle preparations were compared with preparations from untrained mice of the same initial body weight (35 g).

The results in the Table show significantly increased tensions and prolonged fatigue times for the 2 muscles following training. No significant differences were found in contraction times, relaxation times or latent periods following twitch stimulation and the results are not presented here. However, the increase in tension developed by tetanus stimulation (55.6% for the biceps brachii and 79.4% for the tibialis anterior) is not proportional to the increase in fibre cross-sectional area (49.0%).

It is suggested that this disproportionate increase in strength is associated with the thickening of myofibrils recently described by MOLBERT and JIJIMA[2] and GOLDSPINK[3]. Presumably this thickening of the myofibrils would not influence the cross-sectional area of the fibres to the same extent as a proportional increase in their number[4].

Mechanical properties of isolated muscle preparations from control and trained mice

		Biceps brachii	Tibialis anterior
Twitch tension	Control	1112.4 ± 70.7[a]	448.7 ± 40.3[b]
	Exercised	1836.5 ± 195.0[a]	785.5 ± 72.6[b]
Tetanus tension	Control	1845.0 ± 121.7[b]	540.6 ± 54.8[b]
	Exercised	2870.5 ± 141.3[b]	969.8 ± 69.6[b]
Half-fatigue time for tetanus (sec)	Control	5.83 ± 0.74[a]	6.00 ± 0.65[c]
	Exercised	8.94 ± 0.34[a]	7.73 ± 0.80[c]

Mechanical properties of isolated muscle preparations from trained and control mice. Each figure represents the mean of 10 muscle preparations. Tensions are expressed as g/g wet weight of the muscle. S.E., Standard error. [a] Significant at 1% level. [b] Significant at 0.1% level. [c] Not significant.

Zusammenfassung. Weisse Mäuse wurden 3 Wochen auf einem endlosen Band trainiert. Ihre Skelettmuskeln zeigten in vitro erhöhte isometrische Spannung und geringere Ermüdung bei tetanischer Reizung. Die Zunahme an Faserquerschnittsfläche dieser Muskeln war relativ geringer als die Zunahme der Kraftentwicklung. Es wird angenommen, dass diese Disproportionalität als Resultat einer Verdickung der Myofibrillen zu deuten ist.

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The Activity of Neurons in the Lateral Geniculate Body During Wakefulness and Natural Sleep

It is well known that the activity of thalamic and cortical neurons shows marked changes in the transition from wakefulness to sleep[1–16]. Much of the previous research on this subject has been conducted in anesthetized or restrained animals and has dealt with the 'slow-wave' phase of sleep. The purposes of the investigations reported here have been: (1) to study quantitatively the changes which occur in thalamic neuronal activity in unrestrained, unanesthetized animals; (2) to include in the study the phase of 'low voltage-fast wave' sleep[12,13,14,16]; (3) to determine the extent of the neuronal territory within which the changes in neuronal activity occur; (4) to develop a method of implantation of microelectrodes which is simple and does not involve the use of metallic cannulae which frequently cause injury to brain tissue and shorting of the connecting leads.

Materials and methods. Microelectrodes made of platinum-iridium wire, sharpened electrolitically to diameters of from 1–5 μ and insulated with glass[17], were implanted in the brain of 7 cats, under barbiturate anesthesia. After implantation the animals were maintained for periods of from 2–8 weeks, and the neuronal activity was studied in the unanesthetized, unrestrained state, in wakefulness or in sleep.

The data analyzed statistically and presented in this report are from 2 of these animals. The first animal was implanted with 3 microelectrodes in the lateral geniculate body, and recordings from 2 of the microelectrodes (400 micra apart) were taken 1 month after implantation. The second cat was implanted with 1 microelectrode in the lateral geniculate and recordings were obtained 15 days after implantation. Quantitative studies were performed

on 30 sec periods of recording from 2 points in the lateral geniculate body of 1 animal, and from one point in the lateral geniculate body of the other, during each stage of wakefulness and sleep. The location of the recording points was ascertained by serial section of the brain and staining by the NISSL technique.

Electrode implantation. The device used for implantation is illustrated in Figure 1. The microelectrodes were connected to a bakelite piston (B), which could travel downwards within a lucite cylinder, 8 mm in diameter, under the command of a screw (S); the lower extremity of the lucite cylinder was connected to a thin teflon tube (T, gauge 18) which penetrated a short distance into the brain and served to maintain the microelectrodes in the proper direction; the lower part of the lucite cylinder and the teflon tube were attached to the bone by means of acrylic cement and remained permanently fixed, so that, as the piston moved down, the microelectrodes were lowered to the desired location in the brain; the upper extremities of the microelectrodes, which were fixed to the bakelite piston, were connected by means of flexible wires to a 'female' plug (K), completely separated from the cylinder structure, so that inserting the 'male' jack (J) into the plug, in order to make connections with the amplifiers, would cause no change in the position of the microelectrodes. The interior of the cylinder and the space between the cylinder and the connecting plug were partially filled with paraffin (P). A longitudinal slit in the wall of the cylinder allowed the flexible wires to move down with the piston and the excess paraffin to escape into the space between the cylinder and the plug.

The whole assembly was enclosed in a block of acrylic cement (A) attached to the skull. Because the piston fitted into the cylinder very tightly and because of the presence of paraffin inside the cylinder, the downward movement of the piston and the microelectrodes was made against considerable resistance and, when the desired location was reached, with the piston tightly held inside the cylinder, supported by the paraffin below and the screw above, great stability of electrode positioning was achieved.

Recording devices. The microelectrodes were connected to amplifiers with adequate imput impedance and low grid current[4]. The EEG was recorded, simultaneously, through a small screw inserted into the anterior portion of the calvarium, and the EMG through a gross steel electrode inserted into the neck muscles. The screw and the steel electrode were connected to 'Tektronix' 122 preamplifiers. One hour before and during recording the animal was in darkness.

The neuronal spikes were separated by means of a pulse height analyzer[18,19] according to 7 amplitude categories: 30–40 μV, 40–50 μV, 50–60 μV etc., the highest amplitude category containing all the sizes above 90 μV. The frequency of neuronal discharge/unit time, in any of these amplitude ranges or in any combination of them, could be determined and continuously recorded by means of an analog computer. All tracings were monitored on oscilloscopes, pertinent data were stored on magnetic tape and permanent records were made by means of a 'Honeywell-Visicorder' oscillograph (Figure 2).

Results. The quantitative data are presented in the Table. It can be seen in this Table that, as the animals pass from wakefulness to 'slow-wave' sleep, the frequency of discharge of neurons is greatly reduced and as they pass into 'low voltage-fast wave' sleep, the frequency of discharge is greatly enhanced. This is true whether one considers the totality of the spikes contained within all amplitude ranges, or whether one considers separately the spikes classified by the pulse height analyzer in the lower amplitude ranges and the higher amplitude ranges. Analyses of variance performed on these results indicate that the differences in frequency of discharge between the 3 states is significant beyond the 0.01 level in all cases.

The differences in neuronal activity between the various stages of wakefulness and sleep can be seen even more strikingly when the following ratios are calculated: spikes/sec during wakefulness versus 'slow-wave' sleep, spikes/sec during wakefulness versus 'low voltage-fast wave' sleep, and spikes/sec during 'low voltage-fast wave' sleep versus 'slow-wave' sleep. These are 7.28, 0.65, 9.30 respectively. They indicate that the neuronal activity during wakefulness and 'low voltage-fast wave' sleep is many times greater than during 'slow-wave' sleep, and that the neuronal activity during wakefulness is about $^2/_3$ of that which occurs during 'low voltage-fast wave' sleep.

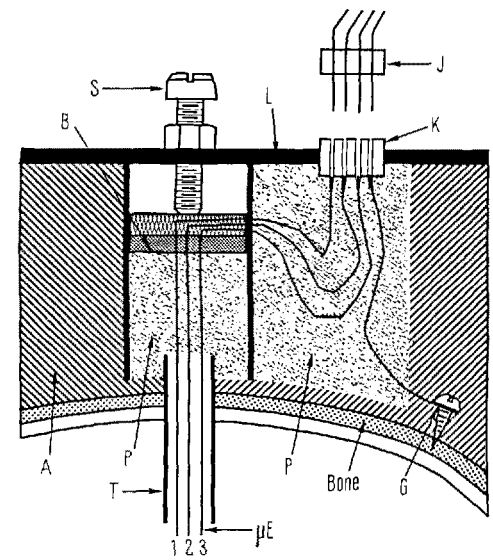

Fig. 1. Diagrammatic representation of device for implantation of microelectrodes. A, acrylic cement; B, bakelite piston; G, 'ground'; J, Jack; K, plug; P, paraffin; S, screw; T, teflon tube; 1, 2, 3 μE, microelectrodes.

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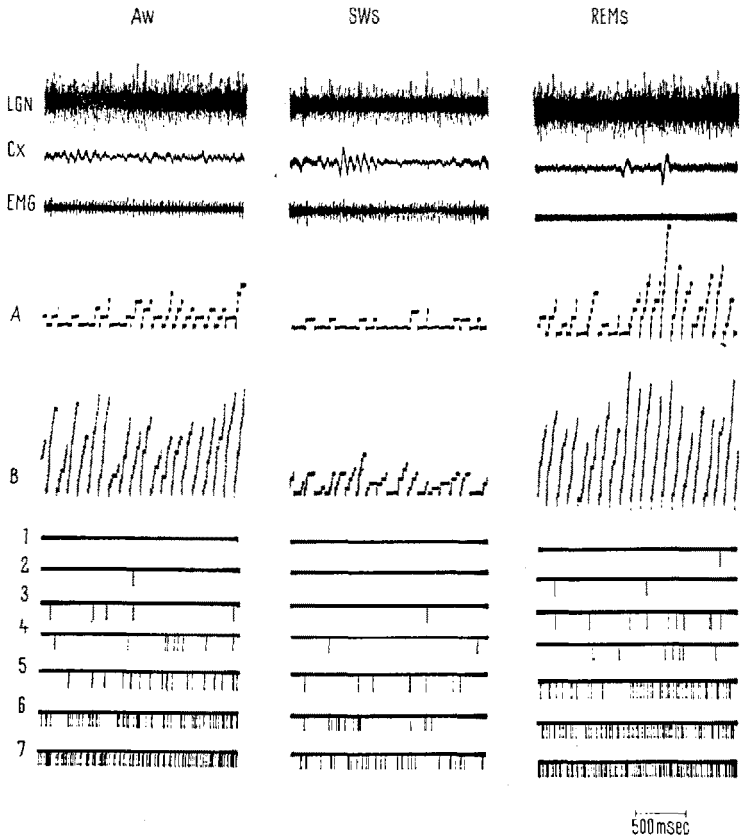

Fig. 2. Original recordings from the unrestrained, unanesthetized cat during wakefulness (Aw), 'slow-wave' sleep (SWs), and 'low voltage-fast wave' sleep (REMs). LGN, tracing obtained with a microelectrode of 5 μ tip diameter showing neuronal spikes from the lateral geniculate nucleus. Cx, electroencephalogram. EMG, electromyogram. The tracings from 1–7 represent the 7 ranges of amplitude into which the spikes shown in the LGN tracing have been distributed by the pulse height analyzer. 1 = 90 μv and above, 2 = 80 to 90 μv, 3 = 70–80 μv, 4 = 60–70 μv, 5 = 50–60 μv, 6 = 40–50 μv, 7 = 30–40 μv. (A) histograms of frequency of discharges having amplitudes avobe 50 μV. (B) histograms of frequency of discharges having amplitudes from 30–50 μV. These histograms were obtained by means of a computer which provided a cumulative record of frequency of discharge of spikes in the LGN tracing every 100 msec. All tracings in each stage of wakefulness or sleep were obtained simultaneously.

Neuronal discharges/sec. Upper figures in each cell indicate spike amplitudes above 50 μV. Middle figures indicate spike amplitudes between 30 and 50 μV. Lower figures indicate total neuronal discharges/sec, at all spike amplitudes. Aw, wakefulness; SWs, 'slow-wave' sleep; REMs, 'low voltage-fast wave' sleep. Cat 1, first animal with recordings from 2 microelectrodes; cat 2, second animal with recordings from a single microelectrode.

	Aw	SWs	REMs
Cat No. 1			
Electrode 1	36.2	8.4	59.1
	127.5	29.2	159.8
Total	163.7	37.6	218.9
Electrode 2	17.2	0.8	26.5
	98.9	7.8	119.5
Total	116.0	8.6	146.0
Cat No. 2			
Electrode 1	16.3	10.0	45.0
	141.7	36.6	208.3
Total	158.0	46.6	253.3

The differences between these ratios are statistically significant beyond the 0.01 level.

In addition, by separating the neuronal spikes according to their amplitudes, it has been possible to show clearly that the changes in the patterns of neuronal activity which occur in the transition from wakefulness through the various stages of sleep, are of 2 kinds: (1) changes in the frequency of discharge of neurons which are active during wakefulness as well as during all stages of sleep; (2) changes in the number of active neurons or groups of neurons with the passage from one stage to another. The first process can be detected by the changes in frequency of spikes which occur within one narrow amplitude range, the second by the disappearance of spikes within certain amplitude ranges in the transition from wakefulness to 'slow-wave' sleep, and by the appearance of spikes within new amplitude ranges in the passage to wakefulness or to 'low voltage-fast wave' sleep.

Furthermore, by recording simultaneously from 2 microelectrodes whose tips were separated by a distance of 400 micra, it has been found that the same changes occur, simultaneously, at the 2 locations (Table). Thus, it has been possible to determine in the unanesthetized, unrestrained animal that the changes in neuronal activity which occur in the transition from wakefulness to the various stages of sleep, or vice versa, develop within relatively large neuronal territories and involve large numbers of neurons[20,21].

Résumé. On a montré que l'activité neuronique du corps genouillé externe du chat diminue lorsque l'animal passe de l'éveil au sommeil à ondes lentes, et augmente lorsque l'animal passe au sommeil à ondes rapides de bas voltage.

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