grossen Teilen der Formatio reticularis lateralis bis etwa 4 mm rostralwärts (Grenze zum Pons) und 2 mm caudalwärts vom Obex ausgelöst werden konnten.

Diskussion. Die Reizserien erzeugen also zweifellos primär Hemmungen der Inspiration. Der inspiratorische Rebound ist ein sekundärer Effekt und wohl am einfachsten damit zu erklären, dass die Inspirationspause auch eine Verzögerung im Aufbau des intrazentralen Hemmungsvorgangs nach sich zieht und dass die Inspiration sich infolgedessen nach der Pause «ungehinderter» entwickeln kann. Eine gleiche Erklärung gibt LÜSCHER[12] für seine Atmungeffekte bei afferenter Vagusreizung beim Kaninchen. Der Hemmeffekt kann sich am Inspirationsbeginn nicht oder nur wenig manifestieren, gewinnt mit fortschreitender Inspiration immer mehr an Deutlichkeit (und mit ihm auch der Rebound) und wird im letzten Drittel vollends dominierend, wahrscheinlich wegen der «progressiven Entwicklung eines zentralen ‹state› während der Inspiration, welcher diese hemmt» (LARRABEE und HODES[5]). Bei Inspirationsabbruch kann sich der Rebound nur noch in einem verfrühten Einsetzen der nächsten Inspiration äussern. Die Reizeffekte während der Exspiration zeigten, dass offensichtlich auch in dieser Phase – zumindest wenn noch ein merklicher exspiratorischer Resttonus vorhanden ist – der intrazentrale Hemmechanismus auf das inspiratorische Substrat noch tätig ist; seine Wirkung kann mit einer kurzen erzwungenen Aktivitätspause des ihn erregenden Substrats vermindert werden.

Summary. In urethane-anesthetized rabbits the bulbar reticular formation was stimulated with volleys of 120 msec duration at 100 pulses per sec, the pulse duration being 0.5 msec. When shifted along the inspiration, the volleys caused an increasingly stronger inhibition followed by a rebound activation of the inspiratory activity. Towards the end of the inspiration, the volleys stopped the latter phase and shortened the following expiration. When applied during the expiration, the volleys also caused an inhibition and shortened this phase. Results suggest that the stimulus-induced pause of the inspiratory activity delays the activation by the inspiratory centre of the central inhibitory feedback mechanism.

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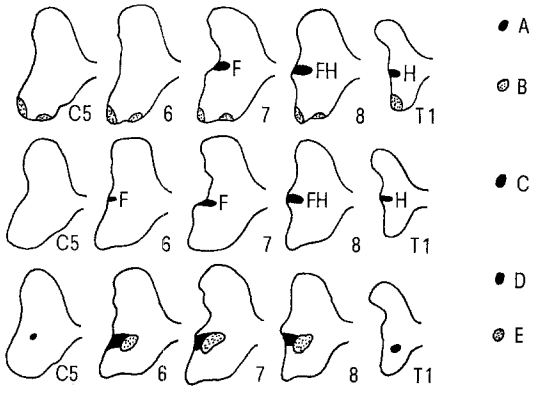
Retrograde Axonal Transport for Cartography of Neurones

Various authors[1–6] have reported experiments which suggest that a retrograde transport, probably axonal, occurs in the nerves. We therefore decided to investigate whether the muscles could be associated by this means with the corresponding motoneurones, and to compare any results obtained with existing chromatolytic maps. Preliminary experiments, using Evan's blue-coupled albumin (EBA) as a fluorescent marker, have already been described in this journal[7].

Materials and methods. The forelimb of Ivanovas 50 albino rats (50–250 g) were investigated. Since the dye tends to spread from the injection site to surrounding areas, only specially selected muscles or muscle groups are suitable. We used the triceps, which is so large that the danger of diffusion into neighbouring muscles is small. The lower arm and paw, which form practically closed systems, innervated by several nerves, were also investigated. The dye was, if possible, injected into muscles innervated by one of the nerves and the others were cut. Only perikarya of this remaining nerve should then be marked. Spread of the marker to other muscles can also be easily seen from the blue colouration and the animal can be excluded from the experiment. The animals were anaesthezised before denervation with Nembutal (Abott; 25–50 mg/100 g body weight). The EBA[4] was then injected as 5–15% solution in saline. The animals were killed 8–144 h after injection and the spinal cord dissected, a needle being stuck in the nerve root C3, for identification. Fixation was carried out in formalin (37% formaldehyde: tap water, 1:3) for about 15 h, followed by washing in water for 20 min. Frontal and transverse cryostat sections, thickness 10 µm and 20 µm, were prepared at a temperature of −18 °C to −20 °C. In the frontal sections, small guide holes were made with a fine needle on the boundaries between 2 segments, before freezing. The sections were preserved either with a mixture of water and glycerol, or with Eukitt after drying. Both methods had disadvantages; with the former fluorescence was diminished after only 12 h, whereas with the latter the structure was generally not so well preserved. For fluorescence microscopy, a Zeiss Universal microscope was used, with a mercury lamp HBO 200, primary filter Schott BG12 and secondary filter Schott 53 + 44.



Cartographical summary of spinal regions in grey matter, where marked cell bodies were found after injection into various parts of the forelimb are indicated A–E. A ●, Muscles of forearm (F) and hand (H) innervated by the ulnar nerve. B ▨, Rhomboideus, Acromiotrapezius and neighbouring muscles of the back. C ●, Muscles of forearm (F) and hand (H) innervated by the median nerve. D ●, Muscles of forearm innervated by the radial nerve. E ▨, Triceps (innervation, radial nerve).

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Segmental distribution of marked perikarya after injection of EBA into different parts of rat's foreleg.

Rat No.	Injection site[c]	Transected nerves	Time from injection to sacrifice (h)	Segments of the spinal cord containing marked motor perikarya[d]				
1	Triceps		8	–	–	–	–	–
2	Triceps		35	–	C6	C7	C8	–
3	Triceps		53	–	C6	C7	C8	T1
4	Triceps		96	C5	–	C7	C8	–
5	Ticeps		144	–	C6	C7	C8	–
6	Triceps	R	30	–	–	–	–	–
7	Triceps	R	96	–	–	–	–	–
8	Hand M	R, U	48	–	–	–	C8	–
9	Hand/forearm M	R, U	80	–	C6	C7	C8	–
10	Hand M, U	M, R, U	48	0	–	–	–	–
11	Hand U	M	30	–	–	–	C8	T1
12	Hand U	M	32	0	–	–	C8	T1
13	Hand U	M	43	–	–	–	C8	T1
14	Forearm U	M, R	80	–	–	C7	C8	–
15[a]	Forearm U	M, R, Mc	85	–	–	C7	C8	–
15[b]	Forearm	M, R, U		–	–	–	–	–
16[a]	Forearm	M, R, U	72	0	–	–	–	–
16[b]	Forearm R	M, U		0	0	C7	C8	0
17[a]	Triceps		12	–	C6	C7	C8	–
17[b]	Hand M	R, U		–	–	–	–	–
18[a]	Triceps		24	C5	C6	C7	C8	0
18[b]	Hand M	R, U		–	–	–	C8	T1
19[a]	Triceps		38	–	C6	C7	C8	–
19[b]	Hand M	R, U		–	–	–	C8	T1

M, Medianus; Mc, Musculocutaneus; R, Radialis; U, Ulnaris. Rat No. 1–14 unilateral injection of EBA; rat 15–19, [a] injection into one side; [b] into the other side of the animal. [c] Injection mainly in muscles innervated by the indicated nerves. [d] negative; C, T, positive; 0, not investigated.

Results and discussion. The first markings appeared in the spinal cord 12 h after injection into the triceps (length of nerve, 30 mm) and 24 h after injection into the hand (length of nerve, 60 mm). Red fluorescence in large motor perikarya (α-motoneurones according to histological criteria) could be observed following unilateral injection only in the ipsilateral ventral horn of the spinal cord. In each cross section there were only 1–4 cell bodies with red fluorescence, the surrounding neurones being generally not marked at all. The marker seemed to be bound mainly to granules in the cytoplasm. With the exception of a few blood vessels, the rest of the spinal cord, namely the dorsal horn, neuropil and the opposite ventral horn were unmarked. (In a few experiments, the injections were carried out bilaterally, but into different muscles.)

In control experiments, in which the injected muscles were denervated, no marked nerve cell bodies were observed in the spinal cord. It can thus be concluded that in our experiments no significant amount of EBA spread to the neighouring muscles, causing unspecific colouration. We have also carried out experiments with uncoupled Evan's blue, using about 1.5 mg/100 g body weight, i.e. about the same amount of pure dye as in the EBA injections. More intensive marking was found in the same perikarya as with EBA. In addition other motoneurones were also coloured, possibly caused by the greater mobility of the smaller molecule in the muscles.

The marked cells were not scattered at random across the ventral horn, but were always in groups. The position of each group, both in the segments and in the transverse sections, was fixed, being dependent on the place of injection and denervation. The Table shows in which segments the colorations were found, and the Figure gives a summary of the results in cartographic form. Regions which have been found to be marked after injection into the back (Acromiotrapezius, Rhomboideus and its surroundings) are also included.

GOERING[8] has used chromatolysis to investigate the pattern of motor cell groups innervating the forelimb in rats. However, as species differences concerning motoneuron positions in spinal cord are small, results from other mammals[9–11] can also be considered. Our results are in general agreement with these investigations. The only difference was found in cranially located marked cell bodies from the median nerve. REED[11] and GOERING[8] found them to be in C_5 and C_7 respectively, whereas our results (for the lower arm only) indicate C_6. From our results, which demonstrate the high specificity necessary for use as a cartographic method, it seems unlikely that an endoneural transport has taken place. In a few experiments using ligation, a weak fluorescence on the distal side of the ligature could be seen. The marker was observed chiefly in the inner part of the axon, suggesting transport inside the axon. Investigations using a more sensitive marker to clarify this point are in progress. In

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larger animals, well-differentiated results can probably be obtained by this method, whereas an extensive chromatolysis can often only be observed when a nerve is severed close to the spinal cord, making a high resolution hardly possible. As a cartographical method, retrograde transport seems to offer several advantages, including the possibility of a combination with other histological methods.

Zusammenfassung. Der retrograde axonale Transport wurde als Methode zur Kartographie von Neuronen bei der Ratte verwendet. Mit Evansblau gekuppeltem Albumin konnten Karten der Perikaria von Neuronen, welche Muskeln der Vorderextremität der Ratte innervieren, angefertigt werden. Die Ergebnisse stimmen mit denen aus Chromatolyse-Versuchen erhaltenen überein.

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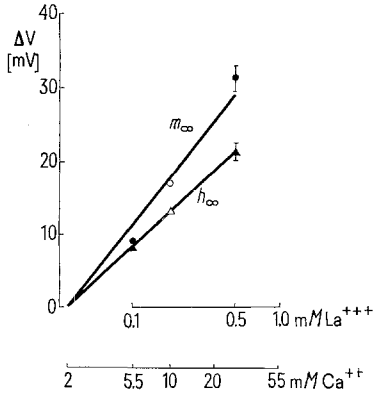
Effect of Lanthanum at the Nodal Membrane

Voltage clamp experiments were done on myelinated nerve fibres of the toad, *Xenopus laevis*. The normal Ringer solution contained 2 m*M* Ca^{++}. The 3 test solutions were: Ringer solution with 10 m*M* Ca^{++}, Ca-free Ringer with 0.1 m*M* La^{+++}, or Ca-free Ringer with 0.5 m*M* La^{+++}. The normal leakage conductance was reduced to 91% in 10 m*M* Ca^{++} and to 73% in 0.5 m*M* La^{+++}; this reduction was not completely reversible. In the sodium current-voltage curves, the negative resistance branches were shifted towards more positive internal potentials and the maximum inward currents were reduced in the sequence (in m*M*): 0.1 La^{+++}-10 Ca^{++}-0.5 La^{+++}. In seven experiments, the maximum sodium permeability was reduced to 80% in 10 m*M* Ca^{++} and to 54% in 0.5 m*M* La^{+++}. The sodium equilibrium potential was not affected.

The steady state values of the activation (m_∞) and the inactivation term (h_∞) of the sodium permeability, according to the Hodgkin-Huxley model[1], were calculated from the current records and plotted as a function of the membrane potential, V. These curves were shifted in the test solutions by ΔV in the depolarizing direction; the shift was completely reversible on returning to normal Ringer solution (2 m*M* Ca^{++}). Cooling from 19 to 8 °C did not significantly influence ΔV. In the Figure the shifts of the activation and the inactivation are plotted versus the logarithm of the Ca^{++} and La^{+++} concentrations. In the range of the tested concentrations, the experimental points can be reasonably fitted by straight lines under the assumption that 1 m*M* La^{+++} be equivalent to 55 m*M* Ca^{++}. In lobster axons the ratio 1:20 has been found[2]. It should be noted that lanthanum, like calcium[3], causes the h_∞-curve to shift less than the relation m_∞ (V).



Voltage shift as a function of Ca^{++} and La^{+++} concentration. Ordinate: voltage shift, ΔV, of the m_∞ or h_∞-curves. Abcissa: Ca^{++} and La^{+++} concentration on logarithmic scales. The lanthanum abcissa has been displaced by a factor of 55 relative to the Ca scale. Open circle: ΔV_m in 10 m*M* Ca^{++} (16.8 ± 0.7 mV, $n = 8$), filled circles: ΔV_m in 0.1 m*M* La^{+++} (9.2 ± 0.5 mV, $n = 5$), and in 0.5 m*M* La^{+++} (31.2 ± 1.8 mV, $n = 5$), open triangle: ΔV_h in 10 m*M* Ca^{++} (13.1 ± 0.5 mV, $n = 12$), filled triangles: ΔV_h in 0.1 m*M* La^{+++} (8.1 ± 0.5 mV, $n = 10$), and in 0.5 m*M* La^{+++} (21.2 ± 1.3 mV, $n = 11$), mean values of 19° and 8 °C. The vertical bars give $\pm$ S.E.M. The slopes drawn by eye to fit the measuring points are 11.0 mV and 8.3 mV per e-fold change in concentration.

Shifts of the permeability parameters have been interpreted as changes in the surface potential of the excitable membrane due to the screening effect of cations on negative surface charges[4–6]. The experimental points of ΔV_m, except that for 0.5 m*M* La^{+++}, which is 10 mV off, can be fitted by curves as calculated for screening from the Grahame[7] equation, assuming 1 electronic charge per 70 Å^2, approximately. Additional binding of Ca^{++} or La^{+++} would require an even larger charge density. For crayfish axons $-1/43$ Å^{-2} has recently been found[8]. The activation curve of the potassium permeability, n_∞ (V), was shifted by 10 ± 1.1 mV ($n = 4$) in 10 m*M* Ca^{++}, corresponding to an approximate charge density of only $-1/200$ Å^{-2} near the potassiun channel. Similar values, $-1/600$[5] and $-1/300$[6] Å^{-2} have been reported, suggesting the effective charge density near the potassium channels to be lower than in the vicinity of the sodium channels.

Zusammenfassung. An der Schnürringmembran sind Lanthanionen etwa 55mal wirksamer als Calciumionen. Die Ergebnisse lassen vermuten, dass die Dichte negativer Festladungen in der Nähe des Natriumkanals grösser als am Kaliumkanal ist.

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