THE ELECTROPHYSIOLOGICAL ANALYSIS OF THE PHENOMENA OF OPTIMUM AND PESSIMUM IN THE NEUROMUSCULAR APPARATUS AT AN EARLY AGE

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Previous work has shown [3, 4] that at an early age in puppies, kittens, and rabbits the phenomenon of the pessimum does not occur, despite the low lability of the neuromuscular apparatus. In these investigations the optimum and pessimum were studied by means of myographic analysis of the mechanism of production of the true pessimum in fully grown dogs.

The object of the present investigation was to study the conditions determining variations in the reactions of the optimum and pessimum in the early stages of development, using an electrophysiological method.

EXPERIMENTAL METHOD

Experiments were carried out on 98 puppies, aged from 1 day to 2-3 months, under light ether anesthesia. The test object was a nerve-muscle preparation (the tibial nerve and gastrocnemius muscle). Electrodes were applied to the dissected peripheral segment of the nerve, 3 mm apart. The nerve was stimulated by rectangular impulses of varying duration (from 0.1 to 1 millisec) from an electronic stimulator. The action potentials of the gastrocnemius muscle were picked up by means of bipolar needle electrodes and recorded on a Disa electromyograph.

EXPERIMENTAL RESULTS

From the results obtained, the experimental animals could be divided into two age groups. The first group included puppies under 16-18 days old, and the second group was composed of puppies aged from 16-18 days to 2-3 mo.

When the nerve was stimulated in the puppies of the first group with impulses of maximal intensity and increasing frequency, the action potentials recorded in the muscle possessed maximal amplitude at frequencies between 5 and 10-12/sec (Fig. 1, a, b). At this frequency, when myographic recording was used, the denticulate form of the contraction curve in the first group of puppies was converted into a confluent curve [8]. This frequency was therefore adopted as defining the lability of the neuromuscular apparatus at this early age. It may be regarded as optimal, because it corresponded to the action potentials of greatest amplitude. Starting from a frequency of 12-15 stimuli per second the amplitude of the action potentials diminished. If the nerve was subjected to prolonged stimulation within the range of these frequencies up to 20/sec for 1 hr or longer, the action potentials of the muscle recorded under these conditions continued with their amplitude unchanged. During prolonged stimulation at these frequencies the muscle showed no signs of fatigue, the criterion of which was the magnitude of the action potentials recorded, even in those cases in which the nerve was stimulated for periods with an average duration of up to 1 hr. The same result was observed when kymomyographic recording was used [5].

If the frequency of nerve stimulation exceeded 22-24/sec when myographic recording was used the following phenomenon could be observed. The contraction of the muscle, having attained a certain height, fell quickly to a particular level, at which it remained in a state of contraction for just as long as the stimulation continued.

The phenomenon we have described in the neuromuscular apparatus of animals at an early age [8], was recorded even earlier by I. A. Arshavskii [2] in the smooth muscle elements of the nictitating membrane during stimulation of the preganglionic fibers of the cervical sympathetic trunk. As a rule, this phenomenon could be recorded only in the

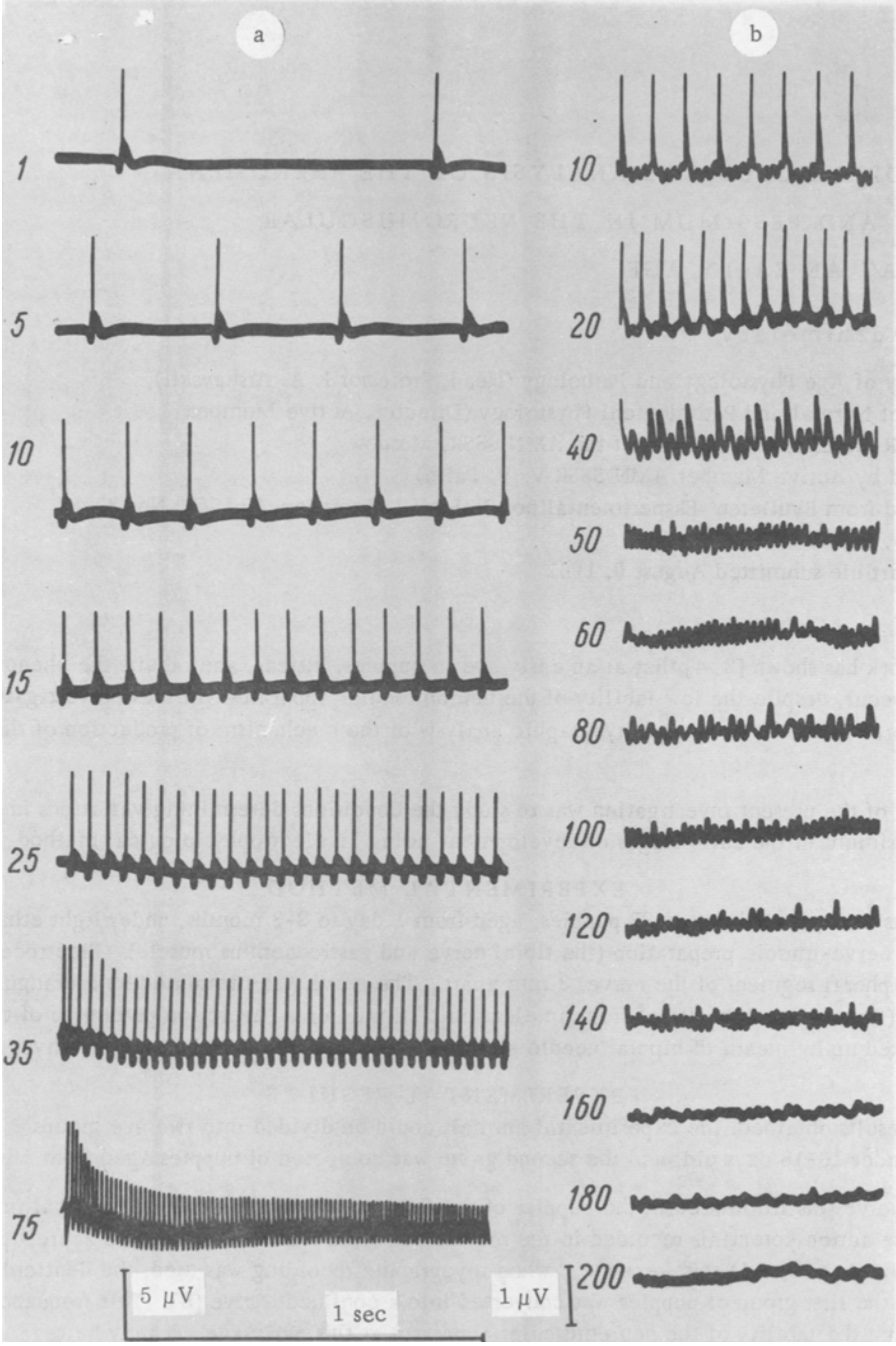

Fig. 1. Action potentials of the muscle of a puppy aged 6 days (a) and of a puppy aged 2 days (b). The numbers denote the frequency of stimulation used.

neuromuscular apparatus of animals at an early age, and in papers published from our laboratory it has been called the "nose" phenomenon.

The same phenomenon has also been recorded during electrographic analysis. It may be seen in Fig. 1, a, that at a frequency of stimulation of 25/sec only the first four action potentials had maximal amplitude, after which, starting with the 7th action potential, their amplitude remained unchanged throughout the whole subsequent period of stimulation. This phenomenon was still more marked at a frequency of 35, and especially at frequencies of 75, 100, and 120-150/sec. The picture, as demonstrated electrographically, made it clear why the "nose" phenomenon was the more marked, when kymographic recording was used, the higher the frequency of stimulation of the nerve.

The electrographic picture we have described was recorded, in particular, in physiologically mature puppies between the ages of 4-5 and 16-18 days. In puppies during the first days of life, and especially in physiologically immature animals, i.e., in puppies with retarded development, in addition to the picture described above a slightly different electrographic pattern could be observed. This took the form of a transformation, initially of the amplitude and subsequently of the frequency of stimulation. In Fig. 1, b, starting at a frequency of 20/sec transformation of the amplitude may be seen and is still more marked at a frequency of 40/sec. After a frequency of 60-70/sec the frequency of stimulation also began to be transformed. Initially, the reproduced frequency of the action potentials was less by one-third, and subsequently by one-half, of the frequency of the stimuli applied to the nerve. At a higher frequency of stimulation the reproduced frequency of the action potentials of the muscle was only between one-third and one-quarter the frequency of the applied stimuli. However, even at a frequency of 200 stimuli/sec action potentials of low amplitude were generated, although considerably transformed, in the muscle. In the puppies of the first age group the action potentials of the muscle almost completely disappeared when the nerve was stimulated with a frequency of 220-250 impulses/sec. The pattern described above was observed during stimulation with impulses not exceeding 1 millisec in duration.

At early age periods in puppies it was difficult to record action potentials of a clearly defined shape, undistorted by artifacts of stimulation (because of the short extent of the nerve trunk exposed by dissection, and, consequently, the short distance between the stimulating and recording electrodes). However, when evaluating the reduced action potentials, distorted by artifacts, we could see that the rhythm of the applied stimuli was transformed not at the myoneural synapse, but in the nerve itself, at the point of stimulation. Transformation at this particular frequency of stimulation can be understood if it is remembered that the duration of the absolute and relative refractory periods of the neuromuscular apparatus is considerably longer at an early age.

Hence, by means of electrographic analysis of puppies at an early age we were unable to demonstrate the power to produce the phenomena of the pessimum, namely the complete relaxation of a muscle and the disappearance of its action potentials, irrespective of the frequency of stimulation applied, within the range of frequencies reproducible by the nerve.

We showed by a special series of experiments that the phenomenon of posttetanic activation [1] cannot take place in puppies in the first age group. We have pointed out that this phenomenon is one of the essential characteristics of the state of the true pessimum. In young puppies, the amplitude of the action potential recordable in the muscle did not exceed that of the initial action potential, irrespective of the duration of the indirect stimulation (Fig. 2).

It was first assumed in 1939 that the myoneural synapse is incompletely formed or absent in the early stages [5], as a result of the discovery that the phase of exaltation is absent and that no pessimum can develop in the neuromuscular apparatus at an early age. At that time the character of the myoneural synapse had not been investigated morphologically in its early stages. In 1940 it was found that in the rat embryo contraction of the muscle in response to stimulation of the nerve appears at the 16th day of development, when no motor end-plates can yet be demonstrated morphologically. In their subsequent researches Soviet and non-Soviet morphologists have shown that the myoneural synapse in the skeletal muscles of animals and man attains its final structural form comparatively late, in the course of postnatal ontogenesis [6, 8, 9, 10, 11].

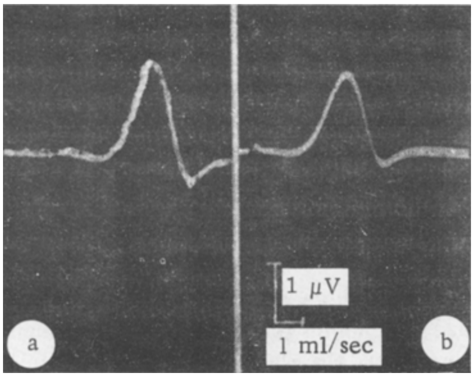

Fig. 2. Action potential of a muscle: initial (a) and after stimulation of the tibial nerve with a frequency of 30/sec (b), in a puppy 5 days old. Recorded by means of a type ENO1 oscillograph.

These findings enable us to understand the reason why a pessimum is impossible at an early age. The absence of structurally formed myoneural synapses may evidently also explain the absence of fatigue, even in those cases in which the neuromuscular apparatus at an early age undergoes prolonged stimulation lasting many hours.

Transformation of the rhythm, brought about by the gradually developing myoneural synapse, may be observed in puppies of the second age group, starting with the 16th-18th day of life. Initially, the action potentials of the muscle disappeared completely at a frequency of 150/sec, later at a frequency of 100/sec, and at the age of 2-3 months – at a frequency of 70-80/sec (Fig. 3). The value of the optimum, i.e., the frequency of stimulation at which the action potentials of greatest amplitude were generated, rose initially to 20/sec, and at the age of 2-3 months to 30/sec. In a puppy 1 month old complete disappearance of the

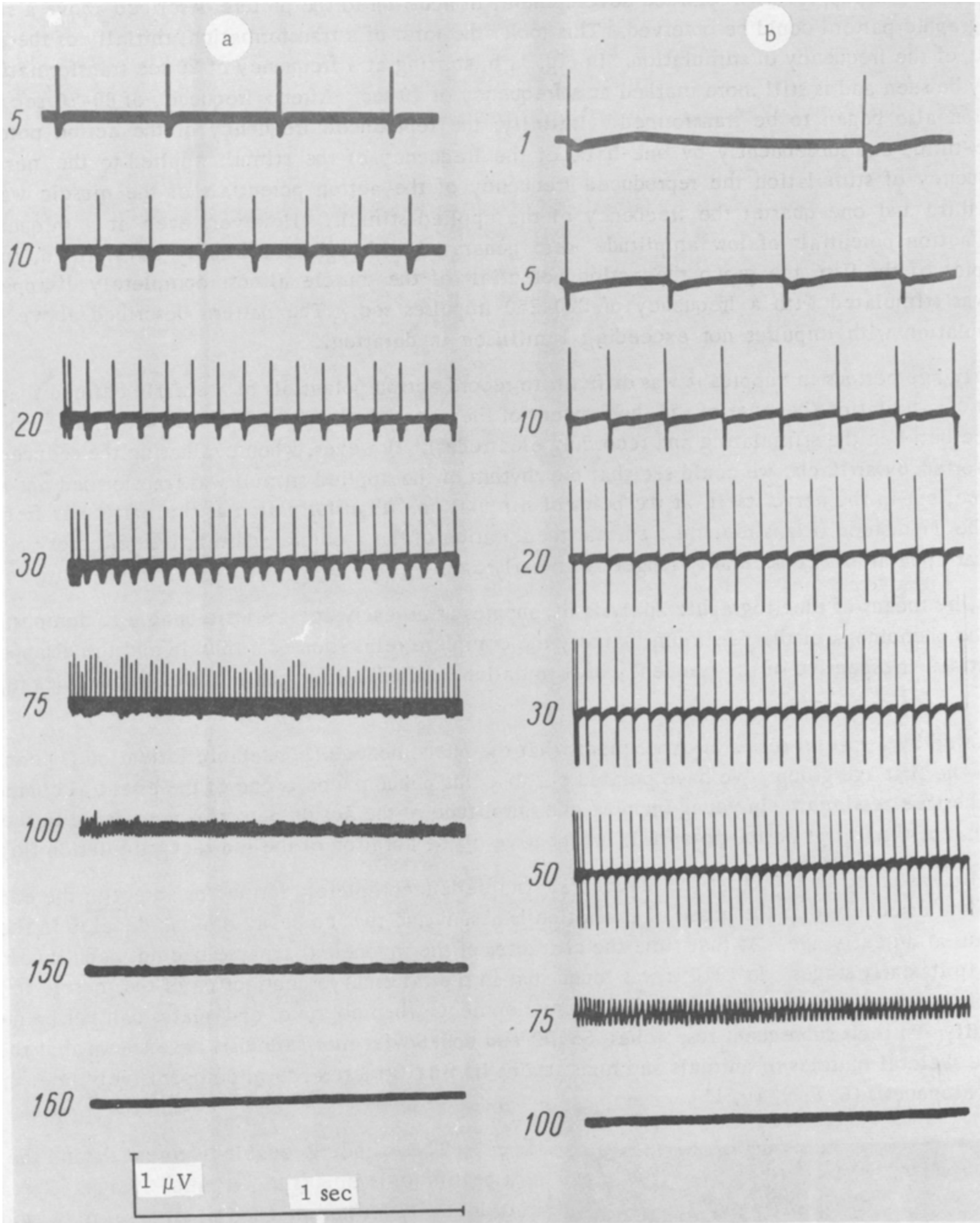

Fig. 3. Action potentials of a muscle of a puppy aged (a) 1 month, and (b) about 2 months. The numbers denote the frequency of stimulation used. Calibration: 10 μV, 1 sec.

action potentials in the muscle took place at a frequency of stimulation of 150-160/sec, and at about 2 months old at a frequency of 100/sec.

Starting from the 16th-18th day of life and thereafter, an ill-defined phenomenon of posttetanic activation could be recorded only in some of the experiments. It was only at the age of 2-3 months that it began to be demonstrable with increasing frequency and clarity. In the puppies of the second age group the electrophysiological manifestation of the "nose" phenomenon had already disappeared at the age of 1 month. Comparison with the action potentials recorded in the nerve suggests that the decrease with age in the limiting magnitude of these potentials reproducible by the muscles is apparently due to the increasing blocking of the nervous impulses at the myoneural synapse. We found that in the puppies of the second age group still characterized by signs of physiological immaturity the pattern of the electrical reaction of the muscle to indirect stimulation closely resembled that described in the puppies of the first group.

SUMMARY

An electrophysiological method was used to analyze specific features in effecting the reactions of the optimum and pessimum in young puppies. In puppies aged under 16-18 days the greatest values of the action potential in the muscle (gastrocnemius) was recorded by stimulating the nerve with a pulse frequency of 5-12/sec, which corresponds to the optimum of the muscle reaction. The pessimum reaction in the animals of the mentioned young age does not take place even when the frequency of the nerve stimulation reaches 200 and more stimuli/sec. In such a case the neuro-muscular apparatus does not show any signs of fatigue in prolonged stimulation, not only by the optimal but also by a higher frequency. The phenomenon of posttetanic activation is not effected at this age. In puppies aged 16-18 days up to the age of 2-3 months there is a gradual rise of the frequency optimum (first up to 20, and then to 30/sec) and a capacity for true pessimum reaction gradually develops.

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