RETICULOSPINAL AND VESTIBULOSPINAL SYNAPTIC INFLUENCES IN KITTENS

D. N. Lenkov and A. I. Shapovalov

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Experiments involving intracellular recording from lumbar motoneurons of kittens of different ages (1-60 days) revealed reticulospinal and vestibulospinal excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials even in animals only 1 day old. With increasing age, the latent period of the monosynaptic type of EPSP shortens progressively, coinciding with a rapid increase in the conduction velocity along the descending tracts.

Bulbar centers exercise motor control mainly through vestibulospinal and reticulospinal projections running in the ventrolateral columns of the spinal cord and possessing a similar structural and functional organization [7,10-12]. The results of a study of motor responses of embryos and newborn animals [1,2,5], as well as data for the myelogenesis of descending fibers [8,15], indicate early maturation of the bulbospinal system controlling posture and locomotion. However, the synaptic mechanisms of suprasegmental influences on motoneurons have been studied only in adult animals.

To ascertain the course of development of suprasegmental control in postnatal ontogenesis, the investigation described below was carried out. Intracellular recording methods were used to study reticulospinal and vestibulospinal synaptic responses of lumbar motoneurons in kittens of different ages.

EXPERIMENTAL METHOD

Experiments were carried out on 40 kittens of three age groups: 10 days, 3-4 weeks, and 6-8 weeks. The animals were anesthetized with nembutal (30-40 mg/kg).

Stimulation of the ipsilateral nucleus of Deiters and nuclei of the bulbar reticular formation was carried out through bipolar electrodes (interpolar distance 0.2-0.4 mm) with square pulses (0.1-0.5 msec, 0.1-2 mA). At the end of the experiment, the electrodes were used for electrolytic marking of the stimulated points. Particularly high thresholds of stimulation (1.0-0.35 mA) were observed in animals of the youngest age group. In experiments on kittens of the middle and older groups, the strength of the stimuli could be reduced to 0.2-0.08 mA.

Because of the absence of sufficiently well-established stereotaxic coordinates for kittens, it was impossible to insert the electrodes accurately in all the experiments into structures giving rise to direct descending tracts (the lateral vestibular nucleus, the caudal reticular nucleus of the pons, the reticular gigantocellular nucleus, and the reticular ventral nucleus). However, as the results of the control histological examination showed (Fig. 1A), provided that stimulation was sufficiently strong, activation of the structures designated was reasonably certain. At the same time, because of the absence of special experiments on animals after preliminary destruction of the vestibular and reticular nuclei, and because of the small size of the brain and the considerable strength of stimulation used in some cases, it was not always possible to differentiate reliably between vestibulospinal and reticulospinal effects.

Intracellular recordings from motoneurons of the lumbar enlargement were made by means of glass microelectrodes filled with 0.6 M K_2SO_4 and having an initial impedance of 5-20 MΩ. The motoneurons were identified from responses to antidromic stimulation of the ventral roots of the spinal cord or to stimulation of nerves to muscles (the nerves to the gastrocnemius and soleus muscles and the common peroneal nerve on both sides) through bipolar silver electrodes or pipets containing Ringer's solution into which the thin nerve branches were sucked.

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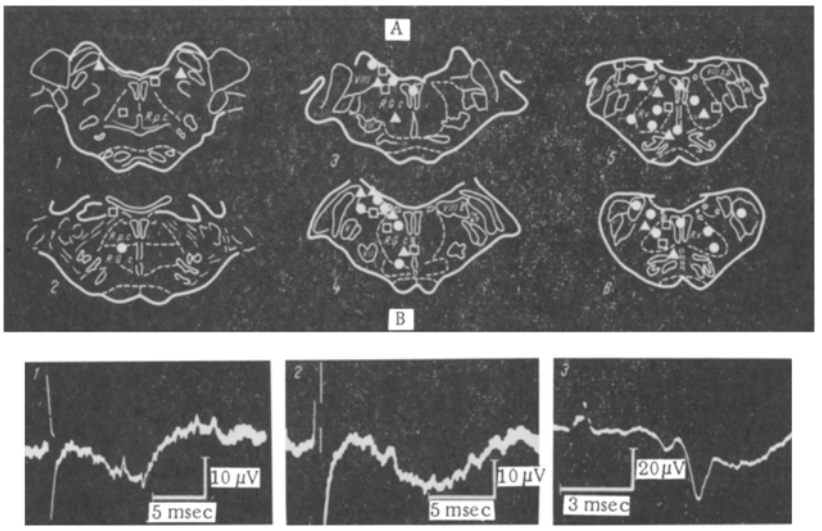

Fig. 1. Localization of points of stimulation (A) and dorsal surface potentials of spinal cord (B) in kittens of different ages. A) 1-6): Sections through pons and medulla in a rostro-caudal direction: squares indicate experiments on kittens aged 1-10 days, triangles on kittens aged 3-4 weeks, and circles on kittens aged 6-8 weeks, R.p.c. caudal reticular nucleus of the pons, R.G.c. reticular gigantocellular nucleus, R.v. reticular ventral nucleus, VIII *l* lateral vestibular nucleus, VIII sp. spinal vestibular nucleus, V nucleus of trigeminal nerve, VII nucleus of facial nerve; B: 1) kitten aged 6 days, strength of stimulation 1.5 mA, distance between points of stimulation and recording 11 cm, 2) kitten aged 24 days, strength of stimulation 1 mA, distance 13.5 cm, 3) kitten aged 2 months, strength of stimulation 0.6 mA, distance 18.5 cm. Downward deflection of beam corresponds to electropositivity.

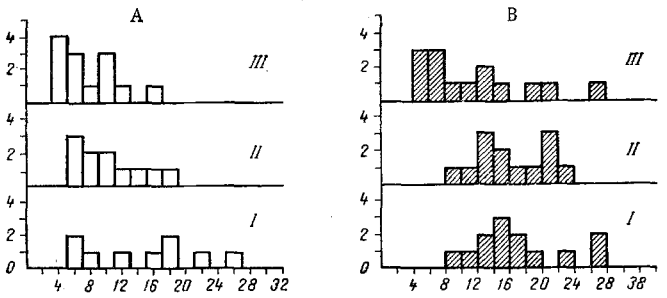

Fig. 2. Histograms of distribution of latent periods of EPSPs (A) and IPSPs (B) of lumbar motoneurons from kittens of different ages in response to stimulation of reticular formation. I) Kittens aged 1-10 days, II) 3-4 weeks, III) 6-8 weeks. Abscissa, latent periods (in msec); ordinate, number of neurons.

To record the dorsal surface potential (DSP) of the spinal cord a unipolar silver ball electrode was used. The recording began after immobilization of the animals by injection of flaxedil or tubocurarine and their transfer to artificial respiration. Motor responses to stimulation of bulbar structures were observed before curarization.

EXPERIMENTAL RESULTS

Application of single stimuli to bulbar structures evoked DSPs in the region of the lower lumbar segments of kittens of all age groups studied (Fig. 1B). Although the amplitude of the DSPs was very small (in the youngest group 10-12 μV), two positive waves could usually be distinguished, the first of short duration (2.5-3, 1.5-2, and 0.6-0.8 msec in kittens of the youngest, middle, and oldest groups, respectively), relatively insensitive to asphyxia, and remaining unchanged during repeated stimulation.

The DSP can thus be regarded as an indication of arrival of a descending wave in the spinal cord along the tracts of the ventrolateral column, and it can be used to measure the velocity of conduction.

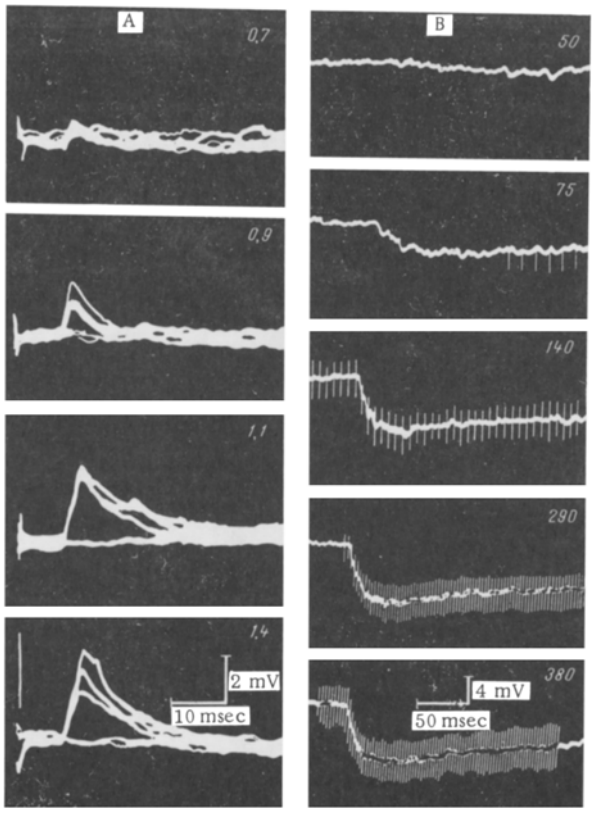

Fig. 3. Vestibulospinal EPSPs of an extensor motoneuron of a kitten aged 10 days (A) and reticulospinal IPSPs of a motoneuron of a kitten aged 1 day (B). Numbers in A denote strength of stimulation (in mA), those in B denote frequency of stimulation (in Hz).

The absolute latent period of the initial component of the DSP fell progressively with an increase in age of the animal, and allowing for lengthening of the descending pathways, this reflects a regular increase in the conduction velocity (Table 1). The change in conduction velocity from 14-16 m/sec in kittens aged 1 week to 50-66 m/sec in kittens aged 7-8 weeks confirms morphological data [8,15] indicating rapid maturation of bulbospinal axons. It was noted that the velocity of conduction along the descending bulbospinal tracts at all age groups studied was somewhat greater than the conduction velocity along peripheral afferent fibers (Table 1), in agreement with results obtained on adult animals [3,4,14,16].

Excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) were recorded in response to stimulation of the reticular formation in kittens up to the age of 10 days in 26 motoneurons, in the middle group in 29 motoneurons, and in the older group in 26 motoneurons. Postsynaptic responses were recorded to stimulation of the vestibular group of nuclei in 5, 19, and 20 motoneurons in kittens of the youngest, middle, and oldest groups, respectively.

In all age periods an EPSP could be obtained in response to single supraspinal stimuli. The amplitude of these responses reached 2-7 mV. During repetitive stimulation, the individual EPSPs usually merged into a continuous depolarization plateau with an amplitude of up to 12-13 mV. IPSPs appeared as a rule only in response to repetitive stimulation, and only occasionally in response to single stimuli.

The minimal latent period of the reticulospinal EPSPs of the lumbar motoneuron fell from 6 msec in the youngest group to 3.8 msec in the oldest (Fig. 2). This last value is very close to the minimal latent period of reticulospinal EPSPs in adult animals [4,6]. Similar results were obtained in experiments with vestibulospinal activation of the motoneurons.

For the most part DSPs and EPSPs of motoneurons were recorded in different experiments, thus complicating the precise determination of the number of synaptic relays along the path to the tested cells on the basis of the duration of segmental delay. Nevertheless, comparison of the latent periods of the DSPs and EPSPs suggests that even in the youngest group of kittens short-latency bulbospinal EPSPs are monosynaptic in nature. Further evidence in support of this view is their rapid temporal course (phase of increase 1.2-2.5 msec, time constant of decline 3-7 msec) and their ability to reproduce high rhythms of stimulation without transformation: up to 100/sec for kittens of the youngest group and up to 200/sec for animals of the middle and oldest groups.

An increase in the strength of stimulation was accompanied by an increase in the response, while sometimes the increase in amplitude of the short-latency EPSPs was discrete in character (Fig. 3A). In some motoneurons, single stimuli caused the generation of a discharge. However, in this case the

TABLE 1. Maximal Conduction Velocity Along Descending Tracts and Afferent Nerves in Kittens of Different Ages

Age of kittens (in days)		6	9	9	24	24	42	42	48	58
Conduction velocity (in m/sec)	Nerve to gastrocnemius muscle and peroneal nerve	10	17	17	21	25	31	40	34	42
	Bulbospinal tracts	16	21	25	27	32	42	32	50	66

possibility could not be ruled out that the cell had been damaged by the microelectrode, because motoneurons in kittens are very small: they do not exceed 20-25 μ in animals aged 2-8 days when measured in specimens stained with toluidine blue.

In the animals of all age groups studied, IPSPs were recorded in response to stimulation of reticular and vestibular structures. Reticulospinal IPSPs of a kitten aged 1 day are illustrated in Fig. 3B. The minimal latent period of the IPSPs was slightly longer than that of the EPSPs (Fig. 2B). During repetitive stimulation of moderate frequency (up to 50-100/sec) reproduction of separate IPSPs to each stimulus was sometimes observed. As a rule, however, the IPSPs were merged into a continuous hyperpolarization plateau with an amplitude of up to 12-14 mV. An increase in the frequency of stimulation not only increased the intensity of hyperpolarization, but also quickened its development (Fig. 3B).

The results thus demonstrate that in early postnatal ontogenesis of kittens, the reticulospinal and vestibulospinal projections, by means of which excitatory and inhibitory synaptic influences are exerted on the lumbar motoneurons, are already functioning. This agrees, on the one hand, with the results of investigation of segmental excitatory and inhibitory synaptic mechanisms of motoneurons, which are apparently formed in the prenatal period [9], and on the other hand, with morphological findings indicating that direct reticulomotoneuronal connections are present in kittens at the age of 20 days [13]. An essential feature distinguishing the postnatal maturation of the bulbospinal connections is the progressive increase in conduction velocity along the descending tract and shortening of the latent period of synaptic responses.

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