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CHAPTER 25

Behavior of hindbrain neurons during the transition from rest to evoked locomotion in a newt

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Introduction

The transition from a resting state to locomotion can be elicited by local electrical stimulation of the midbrain 'locomotor region' (MLR) (Shik et al., 1966). The MLR projects to the spinal cord via hindbrain neurons, at least in mammals (Orlovsky, 1970a). A portion of the hindbrain neurons increases their firing during walking both in the cat and in the rough skin newt (Lowry et al., 1996).

A state transition may arise due to neuronal interaction at the level of the hindbrain when the input from the MLR reaches the threshold for locomotion, similarly to transition between two different rhythmic motor patterns (cf. Green and Soffe, 1996). It can also result due to the shift of individual properties of some neurons or their connections induced by the MLR input.

The aim of this study was to monitor the behavior of hindbrain neurons during the initiation of locomotion. Experiments were performed on rough skin newts. The parameters of the train of stimuli applied to the MLR were near the threshold for locomotion; the latency of movements was about 10 s. This allowed us to record the impulses of single neurons before the motion started. The discharge of a neuron was recorded during one or a few successive trials. If the intensity of stimulation is just threshold for locomotion, one can expect that mainly those neurons that participate in the initiation of this motor pattern would be affected. Part of this study was reported in abstract form elsewhere (Bar-Gad et al., 1995).

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Methods

The spike trains of 44 neurons in the hindbrain of five rough skin newts *Taricha granulosa* were recorded in 10 experiments. The animals had total body length 14 to 17 cm and weighed 12 to 15 g. The animals were immersed in 200 ml of water with 50 mg of MS 222 (Sigma). After 20 min, ice was added. When spontaneous movements as well as tactile reflexes disappeared, a parieto-occipital craniotomy was done. The animals were kept in the refrigerator at 7°C. The first experiment was performed one day after surgery.

The head was fixed while the body was placed in the bath filled with the cool water. The monopolar stimulation was delivered through a microelectrode introduced by a manual micromanipulator under visual guidance. The electrode consisted of a carbon fiber of 7 μm diameter with glass insulation. A train of negative pulses of 1 ms duration with interstimulus intervals from 80 to 120 ms was applied through a constant-current stimulus isolation unit, and the mesencephalic low threshold (3 to 12 μA, 6 μA in an average) point for initiation of locomotion was found. Usually it was located 1–1.5 mm rostral to the caudal border of the tectum, 0.6–0.8 mm from the midline, at a depth

0.7–1.0 mm below the dorsal surface of the brain. Intervals between successive trials were not less than 2 min.

The action potentials of neurons were recorded extracellularly with a similar microelectrode installed in the ipsilateral hindbrain, 0.2 to 0.5 mm from the midsagittal plane using an independent micromanipulator. Recordings were made during the 10 s period before movements started, when near threshold train was delivered. The action potentials were usually biphasic. The action potential width at half-amplitude was typically about 1 ms in the first wave and nearly 2 ms for the second wave. The output of an AC amplifier, bandpass 0.1 to 5 kHz (A-M Systems) was connected to the Board AT-MIO–16-L–9 (National Instruments). After sampling at 10 kHz and storing the data on the hard disk of the PC, the action potentials of particular neuron were identified off line by the threshold ('window') discriminator and checked by their shape. Usually units with amplitudes three or more times higher than the mean level of noise were recognized. Discriminated spike train and the corresponding train of stimuli were then transformed to point processes. Interstimulus intervals (ISIs) were in the range 60 to 1000 ms, usually 200 or 100 ms. A firing ratio (FR) was defined as ISI/μ(I), where average interimpulse interval $\mu(I) = [\Sigma I_k]/n$, $k = 1 \ldots n$, n – number of interimpulse intervals in record.

Results

Direct responses

Action potentials of a neuron whose axon or body was excited by electrical pulse in the midbrain can be recorded at the hindbrain level. These direct responses (11 neurons) had latencies (L) in the range 3 to 15 ms with a standard deviation σ(L) from 0.2 to 0.5 ms at FR < 1. The median coincided with mean latency $\mu(L) \pm 0.2$ ms.

In records with long latencies, drift of latency could be observed during stimulation with ISI = 100 ms and sometimes even 200 ms. The value of drift could achieve 2 to 3 ms during 10 s stimulation by threshold current, and σ reached 0.6 ms. Nevertheless fluctuations around the sliding average remained 0.1 to 0.2 ms (Fig. 1B). Therefore, we considered these as direct responses. Latencies of late direct and early synaptic responses overlapped, but the latter varied in the characteristic span of 2 to 4 ms (Fig. 1C, the early mode).

Direct responses persisted even when responses with longer latency and higher standard deviations could no longer be evoked. The distance between sites of stimulation and recording was 2 to 3 mm, and the conduction velocity was in the range 0.2 to 1.0 m/s. The latency of direct responses remained the same at ISIs 1000 to 60 ms. These responses could be recorded either from the dorsal (granular) or ventral (funicular) layer of the hind brain (Fig. 1A).

Synaptic responses

Non-direct, i.e. synaptic, responses can be subdivided into three groups: time locked to stimuli, non-locked and composite ones. The data presented below are derived from 37 records, each of which contained 17 or more impulses (34 impulses on average). Responses remained unclassified when only a few impulses were generated during 10 s of stimulation.

The response is termed time-locked if the impulses arise in a limited span of ISI and non-locked if they are distributed throughout the ISI. In composite responses, time-locked and non-locked components coexisted. The time-locked component expressed as narrow peak (1.2 to 4 ms wide) in the ISI histogram was defined as a mode. Short (mode < 20 ms) as well as long latency synaptic responses could be recognized among time locked impulses.

Time locked responses

Pure time locked responses occurred in six neurons. All of these neurons were silent. Poststimulus histograms (PSTH) contained both early and late modes, e.g. Fig. 1C, or only the late one as in Fig. 1D, dashed line. In superposition of several histograms, there were two peaks, one at 17 ms, and one at 28 ms. The early responses could alternate with the late ones in the same record. As a rule, the late

response arose after those stimuli in the train that did not evoke the early discharge. This suggests that the threshold of firing is enhanced during a certain epoch after discharge.

Composite responses

Composite responses were observed in 13 neurons, five of them with background discharge (BD). Some neurons generated composite responses and either pure time locked or non-locked responses depending on the parameters of stimulation. Fig. 2A shows that the modes persisted throughout the trial, and the deviations in latency were due to the non-locked responses (Fig. 2C) or BD (Fig. 2B). In fact, the mean interimpulse interval $\mu(I)$ during stimulation (Fig. 2D) became larger than it was during BD in this neuron.

Superposition of histograms of composite responses contains a hump between 18 and 31 ms. In individual histograms either narrow peaks or broad humps could occur apart, and the median of latency was usually 10 to 20% shorter than $\mu(L)$. The relative expression of the time-locked and non-locked components was diverse in different neurons, and in the same neuron the proportion of non-locked impulses could be enhanced when ISI was shortened from 200 to 100 ms (Fig. 3A, C).

Early and late firing

The PSTH for pure time-locked responses was commonly bimodal. There was a late time-locked component in composite responses whereas the early mode rarely did.

Huge deviations of latency occurred in the neuron presented in Fig. 4 during stimulation at ISI = 60 ms (A, D), but not at 100 and 200 ms (A–

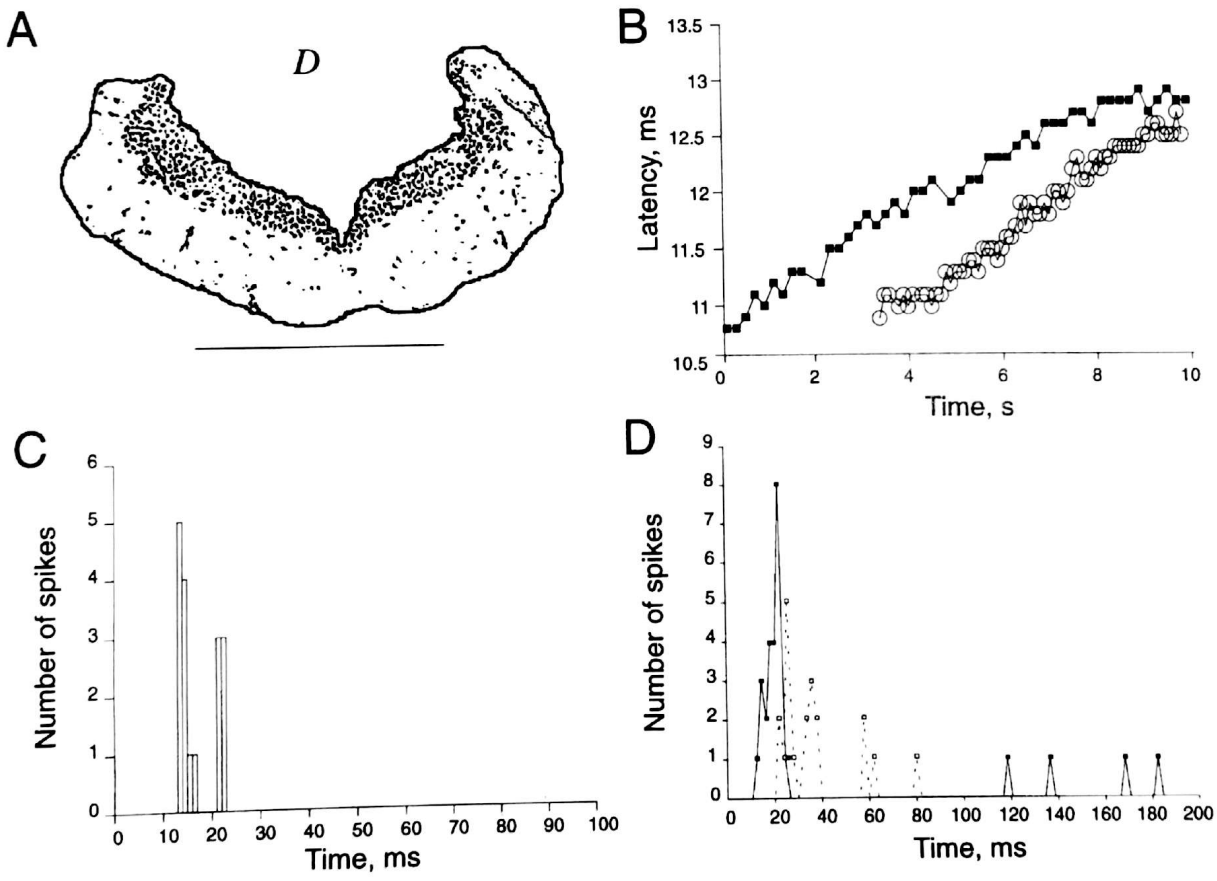

Fig. 1. A: Frontal 10 μm section of the hindbrain (Nissl staining). The bar corresponds to 1 mm. D – dorsal. B: Drift of latency of the direct response in the slow conducting axons. Abscissa indicates time of stimulation, ordinate – latency. Neuron 79A (filled squares) responded to stimuli delivered at ISI = 200 ms, FR = 0.96. Neuron 84A (open circles) fired during stimulation with ISI = 100 ms, FR = 0.97. C, D: Poststimulus histograms of the synaptic responses. Abscissa designates time after stimulus. C: neuron 13A, FR = 0.37, current 3.5 μA, bin 1 ms, only first 100 ms of ISI = 200 ms are presented. D: bin 2 ms. Solid line (black squares): Neuron 64A, ISI = 200 ms, current 6 μA, FR = 0.56. Dashed line (open squares): Neuron 62B, ISI = 100 ms, FR = 0.56.

C). In other neurons, deviations from the normal latency could be observed even at ISI = 200 ms (Fig. 2A–C). These deviations occurred both at low and high FR. Sometimes it was difficult to decide if the late firing was time locked or distributed in ISI (Fig. 1D, solid line, and 3C) or to distinguish uni- and bimodal responses (Fig. 3D).

Nevertheless in 11 records two or even three distinct modes could be identified. The distance between adjacent modes varied from 2 to 12 ms, and the average difference was 5.1 ± 2.2 ms. Together with 12 unimodal records, 38 modes ranged between 12 and 116 ms. Thirty of these modes were less than 33 ms, and their distribution, in turn, included modes at 18, 23 and 28 ms (Fig. 3B). Hence both distances in bimodal histograms and the distribution of modes in records of different neurons indicate that there is a characteristic minimal span near to 5 ms between consecutive latencies. In fact, sometimes there were also the earliest latency responses approximately at 13 ms.

Background discharge and non-locked responses

The distribution of interimpulse intervals in the background discharge (BD) was unimodal and somewhat skewed to the longer intervals, so that usually, the median was less than μ(I). The duration of I_n was almost independent on the duration of I_{n-1}, and the correlation coefficient was less than 0.2. Typically σ(I) was enhanced from 30 to 190 ms when μ(I) increased from 80 to 250 ms and reached 340 ms at μ(I) 500 ms. Correspondingly CV(I) increased from 0.4 to 0.7. This relationship, although variable, was common for BD of different neurons, as well as the distinct states of the same neuron (Table 1). The points corresponding to both non-locked and composite responses were intermingled with BD points on the plane [μ, σ].

Non locked responses exhibited μ(L) of about ISI/2 and σ(L) of nearly ISI/4. Neurons with BD could show either non-locked or composite responses. Usually, both μ(I) and σ(I) of BD

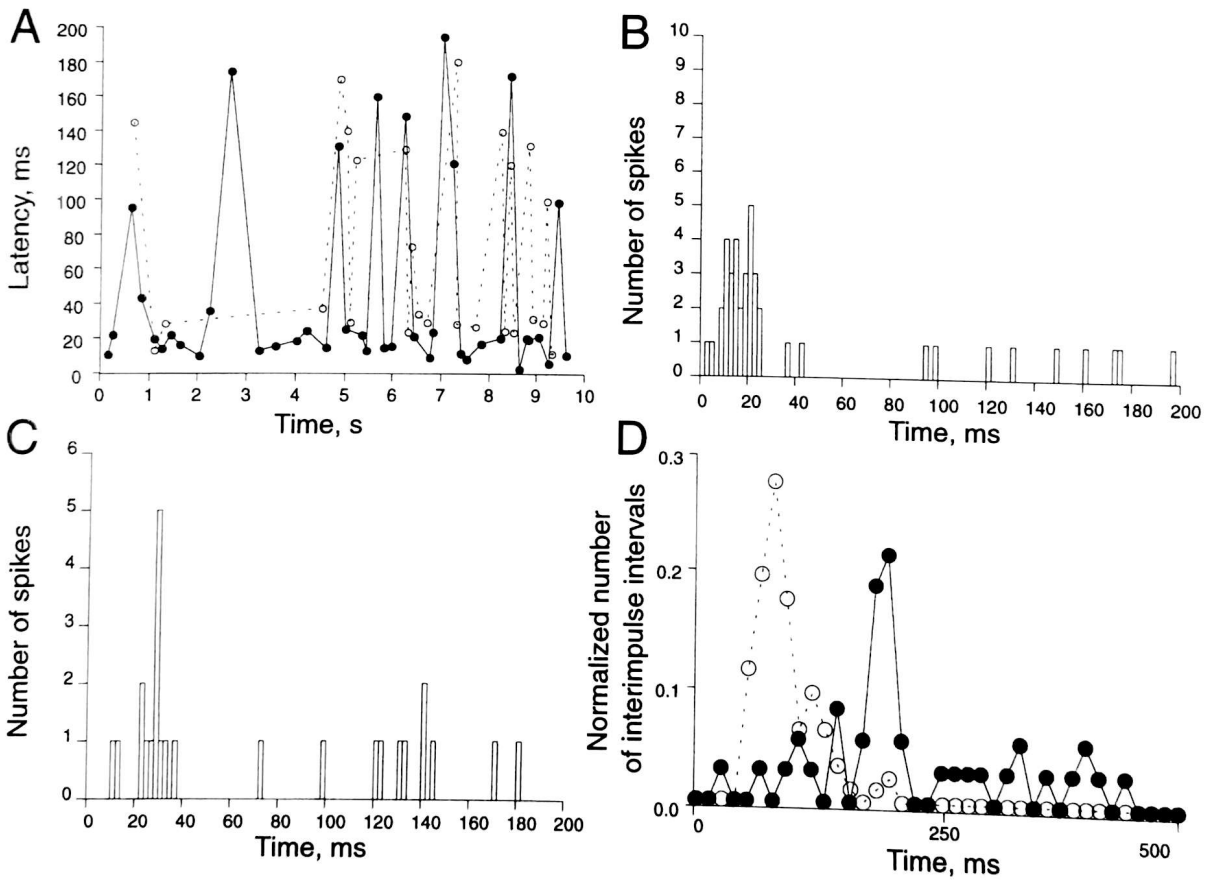

Fig. 2. Composite responses. A: Fluctuations of latency (ordinate) in time course of the trial (abscissa). The same records as in B (solid line, filled circles) and C (dashed line, open circles). B, C: Distribution of impulses throughout ISI = 200 ms, bin = 1 ms. B: Neuron 21A with BD. C: Silent neuron 27A. D: Neuron 21A, normalized histograms (40 bins) of distribution of interimpulse intervals during BD (dashed line, open circles, n = 101) and during stimulation at ISI = 200 ms (solid line, filled circles, n = 42).

diminished during stimulation, but in one neuron $\mu(I)$ decreased while $\sigma(I)$ remained the same. Sometimes $\mu(I)$ remained unchanged whereas $\sigma(I)$ increased. There was also one neuron in which both $\mu(I)$ and $\sigma(I)$ increased when the stimulus train was delivered.

The probability of firing of non-locked responses in silent neurons diminished when the ISI was increased from 200 to 1000 ms. The distribution of interimpulse intervals was similar to that observed during BD of other neurons (Fig. 2D, dashed line). Impulses could be slightly bound to stimuli at the threshold for locomotion, but were distributed in ISI when lower currents were applied. The diminution of the current was followed by a decrease of the firing rate.

Histograms of intervals between impulses in non-locked responses of both silent neurons and neurons with BD were usually unimodal and slightly skewed to the right. Diminished μI during non-locked responses of neurons with BD could remain unrelated to ISI. Distribution of intervals between impulses for the composite response sometimes deviated from the BD pattern.

Rate of stimulation

Three parameters of the train of stimuli effect the threshold for locomotion: the duration of stimulus, the current strength and ISI. They are interchangeable over a certain verge for both initiation of locomotion and single neuron responses. However, the effects of changing the parameters vary for different neurons. The synaptic response of most neurons did not appear at ISI = 1000 or 500 ms, but did appear at ISIs of 200 or 100 ms. To evoke firing at 1000 or 500 ms, it was usually necessary to deliver paired shocks with a 20 or 30 ms-interval between them. A few neurons that responded to single pulses at ISI = 1000 ms increased their FR significantly at ISI = 200 ms (e.g. the silent neuron in Fig. 4B increased its FR by 50% to 0.76). Several neurons generated fewer than 10 impulses

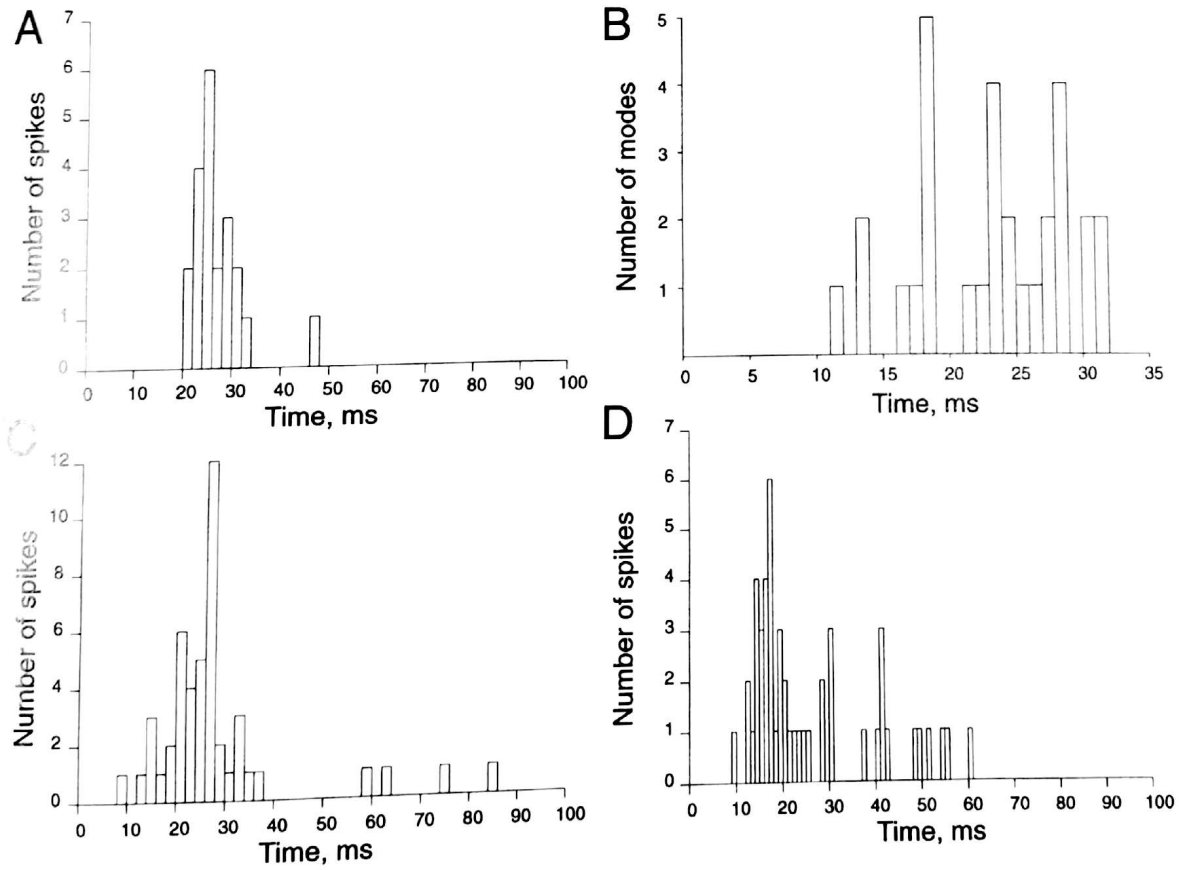

Fig. 3. A, C: Time locked (A) and composite (C) reactions of the same neuron. Distribution of impulses in ISI = 200 ms (A, only first 100 ms are presented) and ISI = 100 ms (C), bin 2 ms. Neuron 64A. B: Histogram of modal latencies < 35 ms, bin 1 ms. D: Obscure response: Neuron 86E, distribution of impulses in ISI = 100 ms, bin 1 ms.

during the 10 s stimulation period even at ISI = 200 ms (FR < 0.2). When ISI was diminished from 200 to 100 ms, these cells showed increased FR. Frequency potentiation was not observed if the neuron has already reached a high FR already at ISI 200 ms.

TABLE 1

Mean interimpulse interval and its CV of the background discharge in four neurons, each being recorded twice

Neuron	Record	μ(I)	CV(I)
21A	21	70	0.21
21A	22	96	0.31
7A	9	205	0.79
7A	7	298	0.87
107A	109	206	0.63
107A	107	424	0.90
14A	17	348	0.51
14A	14	356	0.46

A neuron could display pure time locke responses to weak pulses at ISI = 200 ms, and ad non-locked component when the current or stim ulus frequency was increased (Fig. 1D, solid line One neuron generated impulses time locked t stimuli at ISI = 200, 100 and 60 ms. The earlies latency mode shifted to the right (Fig. 4B–D) in thi cell. In the latter record, a non-locked componen appeared as well.

Stimulation that was subthreshold for locomo tion commonly evoked the time-locked response in neurons with low FR. When the stimulus train reached the threshold, non-locked componen developed, both FR and μ(L) increased, and σ(L was enhanced. The behavior of almost every neuron had its peculiarities. For example, one silen neuron did not respond to single pulses of 4 μA delivered with ISI = 200 ms. An increase in the current to 5 μA was followed by an increased FR and a shortening of μ(L). The average latency and FR almost did not change when pulses 5 μA were

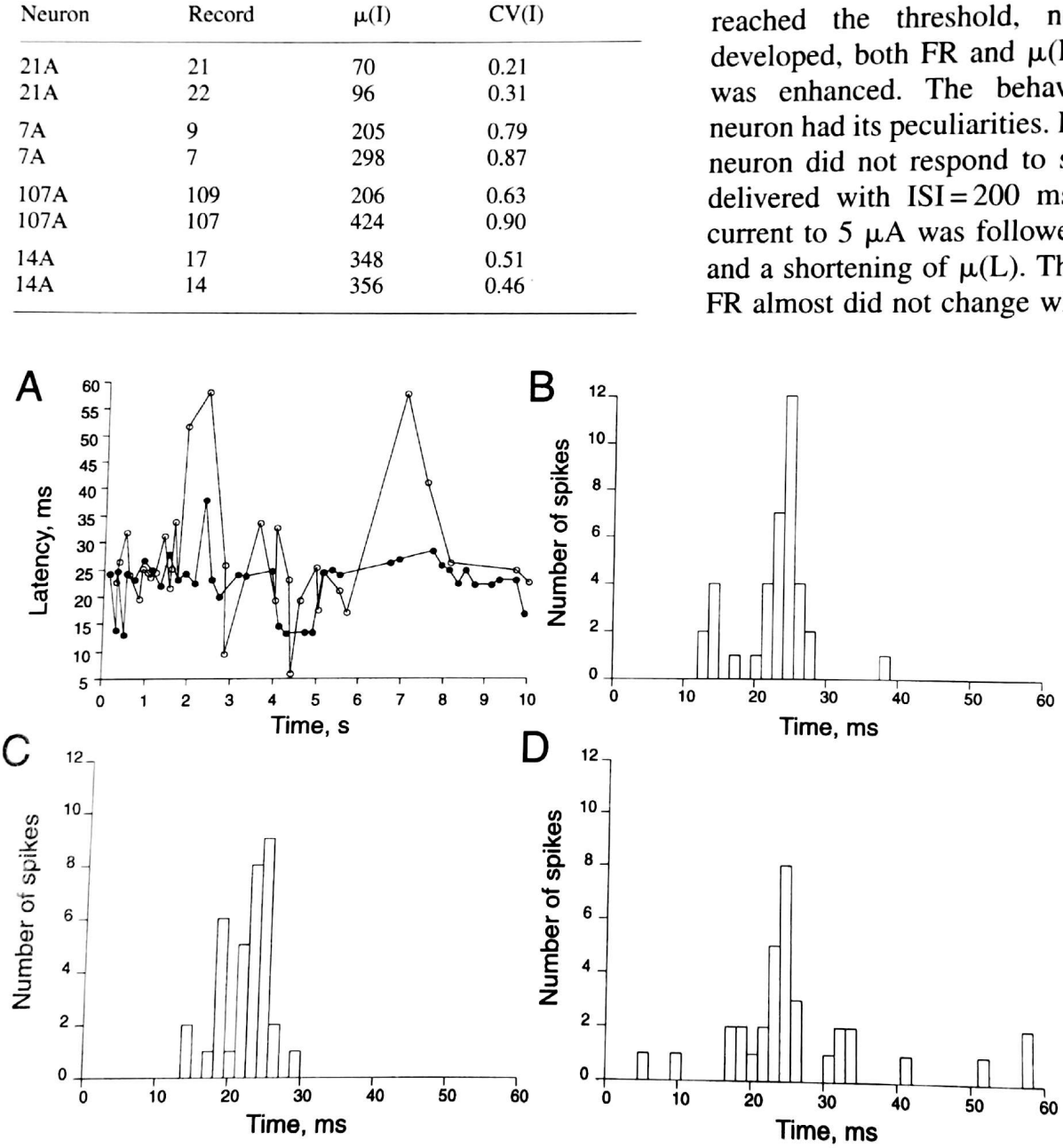

Fig. 4. Latency of the response to stimuli 3.4 μA applied at ISI = 200, 100 or 60 ms. A: Neuron 98A: Fluctuations of latency (ordinat ms) during a train {abscissa, s) at ISI = 200 ms (filled circles) and 60 ms (open circles). B-D: Distribution of impulses throughout IS the first 60 ms are shown. Responses at ISI = 200 ms (B) and 100 ms (C) were pure time locked, and it became a composite one ISI = 60 ms (D), FR = 0.76, 0.35, and 0.20, respectively.

applied with ISI either 200 or 100 ms (Fig. 3A,C). Late impulses appeared at ISI 200 ms when current pulses 6 μA were delivered (Fig. 1D, solid line).

The reproducibility of the neurons' responses seems to be an individual feature of neuronal behavior. A few neurons gave similar responses even though the ISI or stimulus current was altered. In contrast, some neurons did not retain either their FR or latency and σ(L) even in two consecutive trials under the same conditions of stimulation.

Time course of firing

The time course of firing during 10 s of repetitive stimulation was stable in a portion of cells while the rate was augmenting or decrementing in others. Most of augmenting neurons produced 6 to 9 impulses in the first 2 s, while decrementing neurons started mainly from 3 to 6 spikes (Fig. 5A). The difference between the number of impulses produced by augmenting and decrementing neurons grew during the trial twice, although the time course of firing in individual records was almost never monotonic.

The average, normalized time course of firing for the whole population of neurons was uniform, but activity augmented in most of records of non-locked responses, while time locked responses occurred usually with decrement. The time course of firing also depended on strength of stimulation. Four consecutive trials are shown in Fig. 5B at currents 8, 7, 6 and 5 μA. The first response was the composite, others were of the non-locked type. At the two highest currents, the number of impulses produced in the last 2 s of the trial was larger than in the first 2 s. The two lower currents were subthreshold for locomotion, and the firing was rather stabile during period of stimulation. Sometimes the rate of BD during stimulation or the FR of time-locked impulses in the silent neuron was cyclically modulated with a period of 4 to 5 s.

Discussion

Polysynaptic propagation of the input volley

The earliest latency of synaptic responses was 13 ms after the stimulus, although characteristic modes occurred at 18, 23 and 28 ms. This suggests that the (minimal) translation time is approximately 5 ms. This estimation is compatible with data on synaptic delay, rise time and a half-width of excitatory postsynaptic potential in amphibians (Babalian and Shapovalov, 1984).

In our experiments near threshold stimuli were used, therefore the translation time was determined by the rise time and half-width of EPSP rather than by the synaptic delay. A similar value was obtained earlier by Matsushima et al. (1989) in a study in which stimuli applied to the optic tectum evoked monosynaptic EPSPs and spikes in the ipsilateral medullary reticular neurons in a toad with minimal

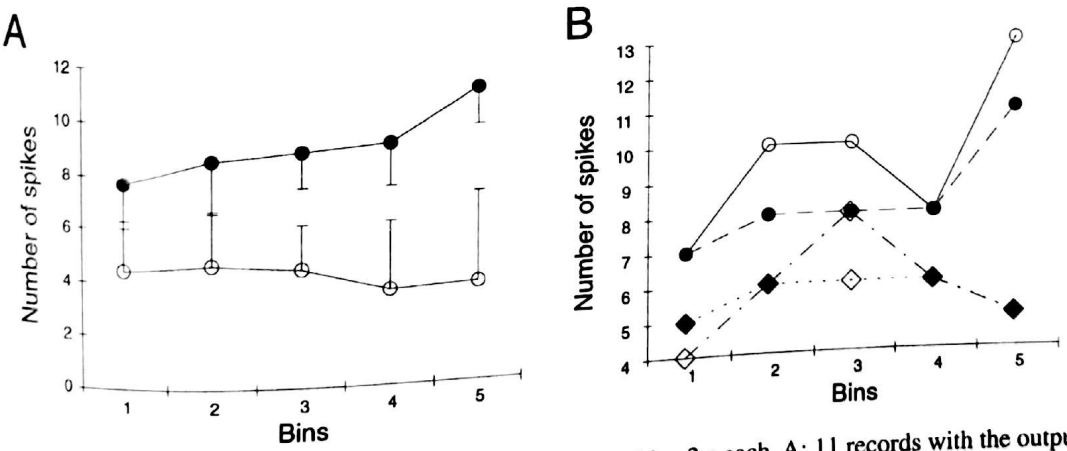

Fig. 5. Time course of firing, abscissa – time, consecutive bins 2 s each. A: 11 records with the output 38 to 51 impulses per 10 s each (filled circles) and 11 records with the total output 17 to 23 impulses (open circles), ordinate – an average number of impulses produced per bin, vertical bars denote σ. B: Neuron 33A, currents 8 (open circles), 7 (filled circles), 6 (filled diamonds) and 5 (open diamonds) μA were applied at ISI = 200 ms. Ordinate indicates number of impulses per bin. Response at 8 μA was composite, others were non-locked reactions.

latencies 2.9 to 3.6 ms and 3.8 to 6.9 ms, respectively. The mean latency of disynaptic EPSPs in cells of nucleus isthmi evoked by stimuli delivered to the optic tract in toads was 16 ± 7 ms (Wu and Wang, 1995).

Since the mode at 13 ms was expressed weaker than later modes and occurred in just a few records, the number of neurons that responded disynaptically was greater than those that responded monosynaptically. This suggests propagation with the coefficient of multiplication > 1 at the second relay. However, the wave-like propagation sharply decrements after 3–4 relays either due to the increased thresholds of neurons that fired in the initial part of ISI, or to the involvement of inhibitory neurons (cf. McLean et al., 1995). In other cases, the activity does not cease but rather becomes asynchronous, in particular if the train achieves the threshold of locomotion.

State transition

Neurons with various rates of BD can coexist at rest because of the paucity of active neurons and their weak interactions. During the transition from rest to locomotion, the neurons must be unified to a certain degree. Perhaps neurons with both decrementing, time-locked responses and augmenting, composite and non-locked responses participate in the transition. The first neurons may start, and those with composite responses then continue processing the input from the 'locomotor region'. Non-synchronized firing of reticulospinal neurons can elicit locomotion without spasticity. The propagation and transformation of synchronous input into this output is executed through polysynaptic interactions between hindbrain neurons.

The state transition means that certain change in the parameters of the input must alter the rules of its processing. Stimulus train applied to the MLR confers on the system the rhythm that might be close to the intrinsic rhythm of a portion of the neurons or neuronal circuits with the corresponding BD. Even if the neurons were silent this input is sufficient to change their average membrane potential slightly, and they will be recruited.

An ample number of medullary neurons discharging at 2 to 7 impulses per second may be necessary to activate the spinal generation of locomotor movements (their characteristic cycle duration ranges between 1 to 5 s). The input neurons and some remote ones in the hindbrain produce impulses that are locked to stimuli when the near-threshold train is applied to MLR. This is a 'laboratory phenomenon' and does not develop under natural conditions of initiation of locomotion. Even when stimuli are applied to one site and all the directly excited neurons fire simultaneously, only a portion of the hindbrain neurons gives synaptic responses that are time-locked to stimuli.

Comparison with the cat

Subthreshold, repetitive stimuli delivered to two different 'locomotor sites' in the feline brain stem together can elicit locomotion, and they can do so whether applied in phase or out of phase (Selionov and Shik, 1991). This suggests that the time-locked responses can be at least partially replaced by responses of other types.

Near threshold (for locomotion) stimulation of MLR evokes EPSPs or short latency impulses only in a small portion of medullary neurons. Most of sensitive neurons give di- or polysynaptic responses both in turtle (Kazennikov et al., 1980) and in cat (Selionov and Shik, 1991), and portion of neurons generate impulses non-locked to stimuli. Frequency potentiation is a prominent feature of the responses of medullary neurons to near threshold stimuli applied to the MLR both in the cat and in the newt. The potentiation is expressed at lower frequency in some neurons than in others. Frequency potentiation enhances the efficiency of weak, distributed input (Selionov and Shik, 1992). Both non-locked reactions and locomotion arise only when the frequency of stimulation is sufficiently high.

Many reticulospinal neurons give off collaterals at the bulbar level both in mammals (Mitani et al., 1988) and in fishes (Metcalfe et al., 1986; Lee et al., 1993). Therefore, these neurons as well as the propriobulbar ones can contribute to interaction at the level of the hindbrain (Kimmel et al., 1985; McCarley et al., 1987). The diversity of the behaviors of hindbrain neurons during the transition between rest and locomotion may be related to

the broad inventory of motor patterns that can arise during stimulation of MLR in urodele (Shik, 1997). There is a wide distribution of properties of individual hindbrain neurons (Rouse et al., 1998; Serafin et al., 1996). Various neurons receive non-identical inputs from different brain stem centers (Shik and Selionov, 1992). Many reticulospinal neurons exhibit stepping rhythm as soon as it arises (Orlovsky, 1970b).

Functional implications

Our data reveal the diversity of the behavior of hindbrain neurons during the transition from rest to locomotion. This diversity can depend on both the individual properties and the position of a neuron in the hindbrain network, in particular on its functional distance from MLR input.

There is a short pathway from MLR to the spinal cord via the reticulospinal neurons (Orlovsky, 1970a). But during the transition period, at least when the threshold train is applied, the processing of the MLR input occurs in the hindbrain. At ISI time scale, multiplication and synchronous translation is executed up to 3–4 relays. Then the wave-like propagation abruptly ceases or transforms into asynchronous activity provided that the train exceeded the threshold. Non-locked firing of these neurons must be transmitted to the spinal cord to elicit locomotion that is not complicated by spasticity.

During the transition epoch, the portion of neurons with composite and non-locked response increases in relation to those with time locked responses, and their firing rates increase. Both the changes in the manner of firing and shift of the latency of the response were observed. We shall report on these and related events in the next communication.

Excitation of a certain number of medial reticulospinal neurons is necessary to activate the spinal locomotor generator from MLR. But the hindbrain is not just a relay station: there is an essential interaction of its neurons, which results in recruiting the reticulospinal neurons appropriate for induction of locomotion rather then postural, scratching or other movements.

Summary

Trains of electrical stimuli were delivered to the mesencephalic 'locomotor region' in the rough skin newt. The current (3–12 mcA) and the interstimulus interval (100 to 200 ms) were adjusted so that locomotion arose in approximately 10 s, or so that the train remained subthreshold for initiation of locomotion. Impulses of single neurons in the hindbrain were recorded during the transition period from rest to locomotion. Time-locked synaptic responses were bi- or unimodal with typical latencies close to 18, 23 or 28 ms, and weak irregular mode near 13 ms.

Impulses that were not locked to the stimuli arose in some silent neurons, and the rate of firing of neurons with background discharge was sometimes enhanced. Composite responses consisted of both time-locked component and impulses distributed throughout the interstimulus interval. The data suggest that short-lived, wave-like propagation of the input volley ceases or is transformed into asynchronous activity after three or four translations. The latter variant could occur if the train reached the threshold for initiation of locomotion. The asynchronous activity persisted throughout interstimulus interval and could coexist with time-locked impulses.

Some neurons generated only a few impulses, while others remained active from beginning to end of the train. These active neurons could either spike at a steady rate, or decrement or augment their rate of firing during the train. The time course of their activity was related to the initial rate of firing. The augmenting type of firing in a subset of neurons may arise due to the interaction of neurons with unstable, steady state and decrementing activity.

Acknowledgments

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