Introduction

To elicit transition from rest to locomotion (rl-transition) in cooled salamander, we delivered the train of electrical stimuli to the mesencephalon and recorded impulses of single hindbrain neurons. Near-threshold train elicited locomotion in about 15 s. Dozens of impulses were recorded during the extended rl-transition.

Methods

The parieto-occipital craniotomy was performed in four salamanders *Ambystoma tigrinum* with total body length 14-18 cm. Before the surgery animals were immersed in cool water with MS 222 (Sigma). Experiments were done one or few days later. The head was fixed, and a glass micropipette with tip diameter 2 ?m filled with 0.5M NaCl was inserted into the midbrain for monopolar stimulation. Train of negative rectangular

pulses of 1 ms duration was delivered with interstimulus interval T 80-200 ms in near-threshold trains and up to 1000 ms in sub-threshold ones. Low-threshold (3-9 ?A) locomotor point was found.

Another micropipette filled with 2M NaCl and impedance 2 M? was introduced in the ipsilateral medial hindbrain for extracellular recording. The distance between sites of stimulation and recording was 4-5 mm. Set of impulses of a neuron was transformed to point series. We considered firing of 15 neurons recorded in 8 experiments. Eighty of these records contained more than 20 impulses each.

Results

The silent neurons that were recruited by near-threshold train (**Fig. 1**) were also involved by sub-threshold ones. Rate of firing commonly decreased during sub-threshold trains (**Fig. 2**) and either increased (**Figs. 4, 6**)

or decreased during near-threshold trains (**Fig. 3**). The number of stimuli k in interimpulse interval I alternated mostly from 1 to 3. Ratio of various k values drifted during the train, or a new k appeared (**Figs. 3, 4**). During near-threshold train, even $k=0$ could arise (**Figs. 4, 5, 6**).

Latency of synaptic responses scattered within two or three ranges that were 5-10 ms apart even during epoch of constant $k=1$ (**Fig. 3**). The modal range could shift gradually (**Fig. 7**) or sharply (**Fig. 1**) during a train. The particular stimulus evoked either short (e. g. 15 ms) or long (e. g. 30 ms) latency time-locked impulse but not both. The time-locked impulses intermingled with delayed ones (**Figs. 3, 7**). The delayed impulses appeared mainly at the middle or the end of interstimulus interval. Time-locked and delayed impulse could form the $k=0$ interval.

Distribution of latency of time-locked responses was uni- or bimodal (**Fig. 7**). Impulses with similar latency, usually time-locked, bordered integer intervals I=kT. Time-

locked and delayed impulses formed non-integer intervals.

Non-integer interval ($k+$) was accompanied mostly by [($k+1$)-] one with difference [I_1 - I_2]<T (**Fig. 5**). Both k and ($k+$) intervals were usually opened by time-locked impulses but the latter were completed mostly by delayed impulse positioned in the middle of ISI (**Fig. 8**). This impulse often started [($k+1$)-] interval, which was completed generally by time-locked impulse. Records that consisted mainly of alternated kT intervals usually included several (+, -) duos (**Fig. 5**).

Trade-off between T and stimulating current in near-threshold trains was not necessarily obeyed by a neuron. The avel usually persisted but the modal latency, occurrence of non-integer intervals, and direction of rate change could differ.

In records of some neurons only a small portion of impulses was time locked and latency had no clear-cut mode. The same neuron generated both time locked and delayed impulses at T=200 ms (**Fig. 3**), but

two or three impulses after every stimulus at T=1000 ms. These impulses were not time-locked to stimuli (**Fig. 2**).

Two neurons with background discharge (BD) increased but one decreased its rate when the train was applied. Impulses were neither time-locked nor phase-coupled to stimuli (**Fig. 9**). Distribution of intervals during BD contained mode and local maxima at two- or threefold intervals (**Fig. 10**).

Discussion

Polysynaptic propagation exhibited discrete ranges of latency. They could correspond to consecutive synaptic translations. Chances of a neuron to be recruited increased during propagation of the wave. However the wave was short-lived and ceased after several translations. The time-locked impulse opened interval, which could be completed unpredictably as either k or ($k+$) one by another time-locked or delayed

impulse, respectively. Ordinarily kT+/- intervals were integer-and-a half when various kT intervals prevailed in the record. But non-integer intervals varied broadly when impulses were scarcely locked to stimuli.

Both propagating wave and phase coupling recruited a neuron by near-threshold input. The presynaptic input depended on the applied train both directly and via its effect on the state of the network. A neuron, which reproduced certain rhythm of stimulation, could not follow the same stimuli applied at the lower rhythm. On the other hand, the train and the volleys of intrinsic origin could result in generation of two impulses in the same interstimulus interval. Impulses of non-locked response of silent neurons originated owe to the indirect effect of input volleys.

Abrupt *k*-transformation, sudden appearance of delayed impulses and sharp shift of modal latency of time-locked responses could arise either together or separately. Various subdivisions of the record by abrupt transformations of dissimilar

parameters were possible. Did different target neurons identify distinct times of abrupt transformation in the same set of impulses?

Summary

Stimuli delivered to the mesencephalic "locomotor region" elicited locomotion in salamander. Near-threshold train of stimuli allowed extending transition from rest to locomotion up to 15 s. The "locomotor region" influences the spinal cord via the hindbrain. Impulses of single hindbrain neurons were recorded during the transition. Occurrence of responses was higher during the train that reached threshold of locomotion than during the subthreshold one. Volleys of the input neurons from the "locomotor region" involved hindbrain neurons in propagating though short-lived wave. Polysynaptic responses appeared more frequently than monosynaptic ones, and consecutive volleys could recruit a particular neuron after e. g. two or three

synaptic transmissions or not at all. The basic variability underlied both pathway of propagating wave and number of transmissions preceding recruitment of a particular neuron at the physiological range of stimulus parameters.

The second common feature was the abrupt transformations. Either number of stimuli between successive impulses or the modal latency could steeply transform. Delayed impulses could appear, in particular around the middle or at the end of interstimulus interval. At the last part of near-threshold train two impulses could arise in some of the interstimulus intervals, one of them time-locked and the other delayed. These events originated due to a developed change of state of the neuronal network rather than as responses to the input volleys themselves. They increased persistent instability of spiking, and subdivision of the set of impulses according to various kinds of abrupt transformations was sometimes equivocal.