Analysis of EPSCs and IPSCs Carrying Rhythmic, Locomotor-Related Information in the Isolated Spinal Cord of the Neonatal Rat

MORTEN RAASTAD,1 BRUCE R. JOHNSON,2 AND OLE KIEHN1
1Section of Neurophysiology, Department of Physiology, University of Copenhagen, 2200 Copenhagen N, Denmark; and 2Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853

Raastad, Morten, Bruce R. Johnson, and Ole Kiehn. Analysis of EPSCs and IPSCs carrying rhythmic, locomotor-related information in the isolated spinal cord of the neonatal rat. J. Neurophysiol. 78: 1851–1859, 1997. To understand better the synaptic language used by neurons in active networks, we have analyzed postsynaptic currents (PSCs) received by interneurons in the isolated spinal cord from neonatal rats during 5-hydroxytryptamine- and N-methyl-D-aspartate–induced fictive locomotion. Using a computer algorithm, we identified PSCs in rhythmically active interneurons in laminae VII and X. To test whether the PSCs actually participated in the transmission of the cyclic, locomotor-related signal, we constructed an analytic current trace based on only the identified events. Each identified PSC was fitted by a mathematical function, and the shape of this function was added to a baseline with time delays given by the time positions of the identified PSCs. By averaging the resulting analytic current trace over several cycles, we showed that the identified PSCs built a cyclic signal locked to the rhythmic activity recorded from the ventral roots. Furthermore, subtraction of the analytic from the original current trace reduced the amplitude of the cyclic signal received by these cells. Thus the identified PSCs contributed to the cyclic information, allowing us to analyze how they built the compound cyclic signal. Most often there was an inverse relationship between the contribution from excitatory and inhibitory PSCs during the cyclic modulation, indicating that there was a reciprocal regulation of the presynaptic inhibitory and excitatory cells. Comparing the most inhibitory and most excitory halves of the locomotor related cycle, there was a considerably larger modulation of the frequency of PSCs than of their amplitude. The small and sometimes insignificant modulation of PSC amplitude suggests that facilitation and depression had little importance for the information transfer. The modest amplitude modification also suggests that the large range of available PSC amplitudes seen in these neurons was not used very efficiently to code the cyclic information.

INTRODUCTION

Understanding the functional organization of a central neural network requires an analysis of the relevant synaptic input that modulates neuronal activity during network function. It has been difficult to study in detail the synaptic information transfer during activity in central vertebrate networks because the necessary experimental conditions and normal network activity are often unobtainable together. We are interested particularly in the functional organization of the central pattern generator (CPG) networks of the mammalian spinal cord because they have the ability, even in reduced preparations, to produce locomotor rhythms that closely resemble those seen in intact animals (Grillner 1981; Rossignol 1996). Such preparations may allow the type of detailed analysis necessary to examine relevant synaptic information transfer in an operating central vertebrate network. We are presently far from understanding these CPG networks because the creation, flow, and cellular integration of the network signals are understood poorly. An important step to understand the mammalian CPG function is to analyze how synaptic signals are used to transmit the cyclic locomotor-related signal. Fast synaptic input, i.e., synapses with ionotropic receptors, could drive cyclic locomotor activity by modulation of the sign (excitative or inhibitory) and by modulation of the frequency and/or the amplitude of the individual synaptic events. Earlier studies have examined the composition of synaptic inputs driving locomotor activity in vertebrate motoneurons by inverting the inhibitory synaptic potentials, either by filling motoneurons with chloride or by hyperpolarizing them. The locomotor CPG network was found to control motoneuron activity by alternating fast excitatory and inhibitory synaptic events during fictive locomotion (Cazalets et al. 1995; Jordan 1983; Perret 1983; Russell and Wallen 1983). Thus there is a reciprocal activity in presynaptic inhibitory and excitatory neurons to drive the motoneurons to fire rhythmically. In this study, we analyzed the fast synaptic signals received by interneurons in the rat spinal cord that were rhythmically active in phase with ongoing fictive locomotion. We asked if the collaboration between excitatory and inhibitory inputs that drives motoneurons was responsible for cyclic activity in a higher level of the motor pathway, interneurons possibly participating in the spinal locomotor CPG. Alternatively, other forms of synaptic signaling could be dominant here. For example, mutual reciprocal inhibition dominates the synaptic signaling in many invertebrate motor CPGs (Marder and Calabrese 1996; Mulloney and Perkel 1988), and a similar organization has been suggested for the rhythm-generating kernel in the mammalian spinal CPGs (Brown 1911; Jankowska et al. 1967; Lundberg 1979; Pearson and Collins 1993; see also Kiehn et al. 1997 for a review).

No matter what the sign of the synaptic inputs, they can influence rhythmicity in the postsynaptic membrane potential either by modulation of the amplitude and/or the frequency of the postsynaptic currents (PSCs). Because little is known about the relative importance of PSC frequency and amplitude modification in functioning networks, we also examined this aspect of the synaptic inputs driving rhythmically active interneurons. If PSC amplitude modification mediates the rhythmic signal, mechanisms for such modulation, like facilitation and depression, will be important to study.
The average PSC amplitude from a population of synapses also could be modulated by a preferential use of synapses with the appropriate strengths, if a range of synaptic strengths were available. This latter possibility is intriguing because the amplitude distributions of postsynaptic currents or potentials in most mammalian neurons reported so far, do indeed show a broad range of amplitudes (see for example Bekkers et al. 1990; Clamann et al. 1985; Mendell and Henneman 1970). On the other hand, if frequency modification of synaptic input is the dominating mechanism in the control of postsynaptic activity, then the factors controlling presynaptic firing rates are important to study.

We used the isolated spinal cord from neonatal rats to examine the synaptic inputs responsible for locomotor activity in spinal interneurons. Under the influence of certain neuro-active compounds, this preparation produces a locomotor-like rhythmic activity that can be monitored as bursts of action potentials in the ventral roots (Cazalets et al. 1992; Cowley and Schmidt 1994; Kiehn and Kjaerulff 1996; Kudo and Yamada 1987; Smith and Feldman 1987). This preparation is well suited for studies of network activity at a cellular level because spinal interneurons in the lumbar region can be recorded from with whole cell patch electrodes (Kiehn et al. 1996).

We concentrated our analysis on rhythmically active neurons located in the intermediate gray matter and close to the central canal because, based on activity dependent dye-labeling studies in cat (Carr et al. 1995) and neonatal rat (Kjaerulff et al. 1994) and spinal cord lesion studies (Kjaerulff and Kiehn 1996), these areas are thought to be important for locomotion. The majority of cells in these areas in the neonatal rat show rhythmic locomotor activity apparently driven mainly by synaptic input rather than by intrinsic bursting/plateau properties (Kiehn et al. 1996). A detailed analysis of how the network uses the synapses for information transfer has been hampered previously by the fact that the individual synaptic events have been difficult to distinguish from each other during ongoing network activity. By voltage clamping the soma, we were able to gather recordings of clearly distinguishable fast synaptic currents that the cells receive and also reduce any contribution of active membrane properties to the rhythmic locomotor related activity.

M E T H O D S

The methods for preparing the isolated neonatal rat spinal cord, inducing rhythmic locomotor-like activity from the cord, and recording the activity extracellularly from the ventral roots and from interneurons intracellularly, using tight-seal whole cell recordings have been described previously in detail (Kiehn and Kjaerulff 1996; Kiehn et al. 1996). Below follows a brief description of these procedures.

Preparation

Neonatal rats (0–3 days old) were anesthetized deeply with ether, decapitated, and the spinal cord extending from C3 to L5 including the ventral and dorsal roots was removed. For better access to spinal interneurons, the cord was split mid-sagittally in most experiments from T12 to L1, and the dorsal side of the cord was discarded. The preparation was transferred to a recording chamber, pinned down, and superfused with oxygenated (95% O2, 5% CO2) Ringer solution of the following composition (in mM): 128 NaCl, 4.7 KCl, 25 NaHCO3, 1.2 KH2PO4, 1.25 MgSO4, 2.5 CaCl2, and 20 glucose (pH 7.4) at room temperature. Locomotor activity was induced by bath application of N-methyl-D-aspartic acid (NMDA, 6–7 μM) in combination with 5-hydroxytryptamine (5-HT, 4–20 μM). The drugs were obtained from Sigma (St. Louis, MO) or RBI (Natick, MA).

Recording

We combined ventral root recordings with intracellular tight-seal whole cell recordings (Blanton et al. 1989; Edwards et al. 1989) from rhythmically active spinal interneurons located around the central canal and in the intermediate gray matter. These are the areas that activity-dependent labeling and lesion studies suggest are involved in rhythm-generation in mammals (Carr et al. 1995; Kjaerulff and Kiehn 1996; Kjaerulff et al. 1994). Activity in the L2 and L3 ventral roots, corresponding to leg flexor and extensor activity, respectively (Cowley and Schmidt 1994; Kiehn and Kjaerulff 1996), was recorded with suction electrodes. Activity in interneurons was recorded with patch electrodes (5–10 MΩ) pulled from 1.5 mm borosilicate glass without filaments. Whole cell voltage or current recordings were made with either Axopatch 1-D or Axoclamp 2B amplifiers (Axon Instruments; Foster City, CA). The signals were filtered at 2–5 kHz and digitized at 5 kHz. Series resistance was followed throughout the experiments and was usually <25–30 MΩ. The pipette solution contained (in mM) 130 potassium gluconate, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 0 or 4 NaCl, 10 ethylene glycol-bis(β-aminoethoxy ether)-N,N,N’,N’-tetraacetic acid, 1 CaCl2, 4 ATP-Mg, and 0 or 0.3 GTP-Li. The pH was adjusted to 7.3 with KOH. Intracellular potentials were not corrected for liquid junction potentials (Neher 1995).

Synaptic detection algorithm

As previously described (Raastad et al. 1996), we used a computer algorithm to detect putative PSCs. In short, an event was considered a PSC candidate if the current deviated from baseline noise more than a specified value within 2 ms. This detection value was set initially so that many events were considered PSC candidates, relative to the final number accepted (see below). An alpha function was fit to the initial 10 ms of the current, to evaluate if the shape resembled a PSC (with an abrupt start, fast rise time and slower decay). The function used was \( f(t) = amp \cdot exp(-\alpha t^2), \) where \( amp \) is the peak amplitude in picoamps, \( t \) is time, and \( \alpha \) the factor characterizing the time course, and the squared deviation between the function and the original data were minimized. The function was defined only for positive values of \( t \), and the current started at \( t = 2 \) ms. The function was fit only to the initial 10 ms of the single PSC because other PSCs and noise often contaminated the PSC tail.

Two measures were used to accept or reject the PSCs: the value of the squared deviation and the peak value of the PSC. These two values were used as detection thresholds set in an interactive (manual) procedure, so that <10% of the outward PSCs detected at depolarized membrane potentials (−40 to −50 mV) were detected at hyperpolarized potentials (−60 to −75 mV). This suggested that the outward events were indeed inhibitory PSCs (IPSCs) (Raastad et al. 1996). This was one of our main criteria for accepting the detected events as PSCs and not noise. Other criteria included visually confirming that the detected events had a shape expected of PSCs and noting that the time courses were different between the outward and inward events (Raastad et al. 1996), which is often the case for inhibitory and excitatory PSCs (Jones and Westbrook 1996). All these features should characterize synaptic events and not noise.

The parameters of the fitted function were stored together with
Synaptic currents giving rhythmic information

Fig. 1. A: 10 s of an intracellularly recorded membrane potential from an interneuron in segment L2 (top) and the concomitant recording of spikes in the L2 ventral root (bottom). Membrane potential is modulated in correlation with the spike activity in the root. B: same cell recorded in voltage-clamp mode shows postsynaptic currents (PSCs) as fast transients in positive and negative directions from a modestly fluctuating baseline. There are more positive transients during high root activity, and more negative transients during low root activity. C: 20 PSCs collected by a computer algorithm (see METHODS) from periods with high root activity (left collection) and 20 PSCs collected during low root activity (right).

The time course of both IPSCs and EPSCs is well described by an alpha-function, and we used this function to identify putative PSCs (see METHODS and Raastad et al. 1996). For example, Fig. 1C shows PSCs detected from the cell in Fig. 1 during low and high root activity. Twenty PSCs were detected during high root activity (first half), and 20 were detected during low root activity (second half). It is obvious that both IPSCs and EPSCs occurred during both activity phases of this interneuron. Most synaptic events detected had a time course expected from unitary PSCs, with an abrupt start, fast and smooth rise time, and a slower decay. This suggests that they were due to synchronized transmitter release from one or more release sites in individual presynaptic neurons.

We first will show that the detected synaptic events actually contained cyclic temporal information phase locked to the cyclic output from the ventral roots. Furthermore, we will show that the detected PSCs actually were a significant part of the cyclic information that a cell received, because the original cyclic signal was reduced significantly in amplitude when these PSCs were subtracted digitally from the current trace. This verification of the detected PSCs as information carriers allowed us to analyze, finally, how the cyclic information was built by the PSCs.

Identification of PSCs giving locomotor-related information

If the detected events carried cycle-related information, one would expect to reduce the magnitude of the total cyclic information a cell received by subtracting these events from

Results

Interneurons receiving cyclic locomotor-related information

Concomitant recordings extracellularly from spinal cord motor roots and intracellularly from cells located close to the central canal or in the intermediate gray matter often showed interneurons with rhythmic membrane potential modulation that correlated with rhythmic ventral root activity (Kiehn et al. 1996). For example, Fig. 1A (bottom) shows 10 s of rhythmic ventral root activity (L2) during pharmacological induction of a locomotor rhythm with NMDA and 5-HT, whereas Fig. 1A (top) shows an interneuron firing out of phase with the ventral root activity. We cannot conclude that this neuron is a component of the spinal network producing locomotor activity, but it obviously received timing information from the locomotor network. The cyclic, locomotor-related information that the interneuron received appears to be mediated, at least partly, by fast synaptic events. These synaptic events can be better seen under voltage clamp, which reduces their duration and makes them stand out as fast outward and inward transients, probably representing IPSCs and excitatory PSCs (EPSCs), respectively (Fig. 1B, top).

The time of the start of the function relative to the locomotor period they appeared in. The start of a locomotor period was defined as the onset of a ventral root burst. PSC charge was calculated as the integral of the fitted function, numerically integrating until the amplitude was <1/100 of the peak value.

The detected PSCs were then tested to see if the total cyclic information a cell received by subtracting these events from the current trace. This verification of the detected PSCs as information carriers allowed us to analyze, finally, how the cyclic information was built by the PSCs.
rhythmic modulation of the whole cell current that is not mediated by fast synaptic transmission.

The cyclic signal in an intracellular current trace is more obvious when the signal is averaged over many locomotor cycles. For this illustration, in Fig. 2B, each cycle was divided into 10 time bins, and the average and SE of the current in each bin was calculated, in this case over 20 cycles. The cycle was defined as the period from the start of the root burst to the start of the next burst, determined by eye from the root recording. The resulting values are expressed as histograms with SE given as vertical lines on top of the bars. The lower amplitude of the averaged signal in the residual current trace as compared with the original suggests that the detected events (the analytic current trace) contained important cyclic information that was similar to the original trace but of lower amplitude (~45% of the original in this case).

We examined whether there was significant cycle-related information in the original and the analytic current traces by testing for a significant difference between the integral (charge) of the first and second halves of their cyclic signals using a Student’s t-test. All 16 experiments included in the following analysis showed a significant ($P < 0.05$) cyclic signal in all traces. Furthermore, there was a significant reduction in the amplitude of the original trace after subtraction of the analytic trace ($P < 0.05$). This means that, despite not accounting for all the locomotor-related information, the identified PSCs were an important contribution to this information. We now can examine the composition of this subset of the cyclic information driving the locomotor-related activity of spinal interneurons.

**Decomposition of the synaptic information**

We next examined the relative contributions of the excitatory and inhibitory synaptic events to the generation of cyclic information. For the rest of the analysis, we arranged the intracellular locomotor-related cycles so that the most inhibitory (positive) phase was always in the first half of the cycle (Fig. 3A, left). We can distinguish three ways the PSCs can build a cyclic signal. The simplest possibility is to transfer the cyclic signal by EPSCs or IPSCs alone. Either a relative increase in the amount of excitatory charge or a relative decrease in the amount of inhibitory charge could make the second half of the cycle more excitatory than the first half. A second possibility is that both the inhibitory and the excitatory charges together form the cyclic signal. This would mean that the excitatory charge increased and the inhibitory charge decreased in the second half compared with the first half. A third possibility is that the amount of both inhibitory and excitatory charge increases simultaneously in one cycle half. In this case, the main cyclic signal would be dominated by either the EPSCs or the IPSCs, whichever contributed the largest charge difference between the two halves of the cycle. The less dominating charge in that case would counteract the main signal. The following analysis will demonstrate that all three possibilities are realized in the cyclic information-driving rhythmically active interneurons.

To analyze the relative contribution of inhibitory relative to excitatory charges to the cyclic signal, we divided the detected PSC populations in two groups according to the sign
The relative changes in synaptic charge comparing the first and the second cycle halves must be due to changes either in the frequency or the average size of the PSCs or both these factors. The total charge contribution can be calculated as the average charge of the PSCs multiplied by their frequency, illustrated in Fig. 4A for the EPSCs and IPSCs from Fig. 3A. In this experiment, the average size (charge) and the number of PSCs occurring within 10 equal time bins during an average cycle show that the size changes very little, whereas there is an obvious modulation in the frequency of PSCs during the cycle.

The relative change in frequency and amplitude was estimated separately for IPSCs and EPSCs for all experiments by dividing the frequencies and the average size in the sec-
A cyclic signal given by EPSCs (top histograms) can be divided into a contribution from modulation of the average PSC size (charge) multiplied by their frequency. Same is done for the IPSCs (bottom histograms). During the average cycle, there is no detectable modulation of the average size but an obvious modulation of the frequency of PSCs. Relative change in average charge and frequency from the first halves to the second halves of the cycles was estimated for all experiments. The results are given as a circle around the mean with a vertical line giving the standard error. ●, differences from 0 with  \( P < 0.05 \). For the frequency estimates, the vertical line gives the standard deviation in a binomial population with  \( N \) equal to the sample size. In those cases, where either EPSCs or IPSCs did not give a significant contribution to the signal, the cell is marked only with a vertical line on the unit line. We can see a more obvious modulation of the frequency of PSCs than of their average size.

In this study, we have recorded from rhythmically active interneurons located in the intermediate gray matter and around the central canal in the isolated neonatal rat spinal cord during transmitter-induced locomotion. Neurons in these areas probably are involved in locomotor rhythm generation (Carr et al. 1995; Kjaerulf and Kiehn 1996; Kjaerulf et al. 1994). The cyclic modulations of interneuron membrane potential correlated with the locomotor associated ventral root activity. The neurons, therefore, received synaptic information from the network generating the rhythm and therefore may have been a part of a locomotor CPG.

We extracted a subpopulation of synaptic events from these neurons that also contained a cyclic signal. The identified PSCs were, indeed, a part of the population of PSCs that transmitted the cyclic information because there was less cyclic information left when the identified events were subtracted. To our knowledge, this is the first example of identified populations of unitary PSCs that carry information from a mammalian network producing a motor output. Such data open the possibility to analyze how the synaptic events built the cyclic signal.

**Detection of PSCs**

The majority of detected events are probably the result of transmitter release from individual release sites or release
from several release sites synchronized by individual presynaptic neurons (unitary PSCs) because of the following observations: 1) the shape was characterized by an abrupt onset, fast and smooth rise time, and a slower decay, similar to unitary synaptic currents in other central neurons; 2) the outward currents were reduced greatly in amplitude, and therefore not detectable, at −65 mV compared with −45 mV (see METHODS and Raastad et al. 1996); and 3) the detected events formed a cyclic signal that was locked to the root activity, which would not be expected from noise or other electrical processes in nerve cells. This last argument strongly suggests that most PSCs appeared in response to presynaptic spikes and not because of spontaneous vesicular release, which would not be expected to build a regular signal.

Characteristics of the whole population of PSCs would be detectable also in this subpopulation if it was representative for the whole population. It is, therefore, important to be aware of the detection criteria. The PSCs were identified based on their shape and amplitude. Because we used an amplitude threshold for detection, there is probably a part of the PSC-population that is smaller than the detected PSCs and therefore not included in the analysis. Amplitude and frequency modification restricted to these small PSCs would therefore not be included in our analysis. The amplitudes of the detected PSCs covered, however, most of the amplitude range of the complete population because the largest 10% were at least four times larger than the smallest 10% in all experiments. This means that even if the smallest, undetected PSCs had amplitudes close to zero, the detected events still covered 4/5 of the amplitude range, giving the opportunity to study both large and relatively small PSCs.

**Contribution from IPSCs and EPSCs to the rhythmic signal**

In most of the interneurons from which we recorded, the cyclic locomotor related signal was mediated by an inversely related contribution from both EPSCs and IPSCs (Fig. 3). This alternating dominance between EPSC and IPSC activity, or push-pull drive (Russell and Wallen 1983), is similar to how the rhythmic network drives the motoneurons in lamprey (Russell and Wallen 1983) and mammals (Cazalets et al. 1995; Jordan 1983; Perret 1983).

Because the neurons recorded from are situated in an area that is particularly active during locomotion (Carr et al. 1995; Kjaerulf et al. 1994) and also seems to be necessary for the generation of the rhythm (Kjaerulf and Kiehn 1996), they actually may be a part of the mammalian locomotor CPG. It is therefore interesting to compare our findings to investigations of and theories for CPG function. Many motor CPGs in invertebrates are dominated by rhythmic synaptic influence from IPSCs (Marder and Calabrese 1996). In these animals, the basic building blocks of the CPGs are half-center oscillators based on reciprocal inhibitory connections. A similar mutually inhibitory half-center organization has been found in mammalian CPGs generating respiration (Bianchi et al. 1995) and has been proposed for the kernel of the spinal network generating mammalian locomotion (Brown 1911; Jankowska et al. 1967; Pearson and Collins 1993; see Kiehn et al. 1997 for a review). Theoretical studies also show that, in a half-center model, reciprocally inhibitory connections in combination with tonic excitation and/or intrinsic membrane properties are sufficient to generate both alternating and synchronous rhythmic activity (Perkel and Mulloney 1974; Sharp et al. 1996; Wang and Rinzel 1992).

Thus the alternating rhythmic contribution from both EPSCs and IPSCs found in the present investigation gives the opportunity for a more complex signaling than that seen in or hypothesized for the CPGs mentioned above. We, however, will emphasize that these data are not necessarily at odds with the mutually inhibitory half-center theory for the CPG. First, we may not have recorded from the cells that actually create the rhythm; they just may receive information from the rhythmic network, like, for example, motoneurons do. If the kernel of the CPG consists of relatively few neurons, it would be unlikely that the neurons we have recorded from are representative for the CPG function. Second, not all our neurons showed an alternation between excitatory and inhibitory contributions to the cyclic signal, leaving the possibility that portions of the network actually could give rhythmic information through either IPSCs or EPSCs alone.

**PSC frequency and amplitude modification**

Although significant modulation in both average amplitude and frequency were seen in the cyclic information transfer, frequency modification of the PSCs was contributing more than amplitude modification to the creation of the cyclic locomotor-related signal. The interpretation of the observed changes in PSC frequency depends on several un-known presynaptic factors. If the synapses in this network typically have few release sites combined with low release probability at the individual sites (as seen, for example, in the hippocampus: Arancio et al. 1994; Gulyás et al. 1993; Hessler et al. 1993; Rosenmund et al. 1993), the increased frequencies of PSCs simply could be due to an increase in release probability. If the increased frequencies were the result of increased presynaptic spike activity, the additional PSCs could come from the same cells that caused PSCs during low frequencies, or they may come from cells that were active only during the high-activity half of the cycles. This is an important distinction for the identification of cells that contribute to the rhythmic behavior, and this could be illuminated by studying spike frequency modification in extracellular single-unit recordings during locomotion.

The interpretation of the significant amplitude modification in some experiments is ambiguous because an increase in frequency could affect the probability for superimposition of more than one PSC. A certain fraction of the detected PSCs are probably the summed effect of more than one unitary PSC, and the frequency of such multunit PSCs probably would increase with increasing frequency. Another possibility is that the increases in mean PSC amplitude represent a true facilitation, with increased release probability at multi-quantal synapses. Even if we accept a contribution from amplitude modification to creation of the cyclic signal, this contribution was modest and sometime lacking. This observation suggests that mechanisms for amplitude modification, like synaptic depression, desensitization and facilitation, are not very important for the information transfer at these cells. It also suggests that the large range of amplitudes that was...
available was not used very efficiently to code the cyclic signal. The absolute value of the largest 10% of the PSC amplitude populations was always more than four times larger than the smallest 10%. One possibility is that the differences in PSC amplitudes were caused by systematic differences between the synaptic connections, for example in their position on the dendritic tree, or in the number of contacts, release sites, or channels. The different amplitudes in this case would be controlled by different presynaptic elements, as we have described in this article, the preparation of central pattern generators. In: Neural Control of Rhythmic Movements, Soc. Exp. Biol. Symp., 37, edited by P.S.G. Stein, S. Grillner, A. Sleverson, and D. G. Tin Vertebrates, edited by A. Roberts and B. L. Roberts. Cambridge, UK: Cambridge Univ. Press, 1993. 423–444.


NEHER, E. Voltage offsets in patch-clamp experiments. In: Single Channel


