In Situ Characterization of a Rectifying Electrical Junction

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Rela L, Szczupak L. In situ characterization of a rectifying electrical junction. J Neurophysiol 97: 1405–1412, 2007. First published December 13, 2006; doi:10.1152/jn.00973.2006. Electrical synapses play significant roles in neural processing in invertebrate and vertebrate nervous systems. The view of electrical synapses as plain bidirectional intercellular channels represents a partial picture because rectifying electrical synapses expand the complexity in the communication capabilities of neurons. Rectification derives, mostly, from the sensitivity of electrical junctions to the transjunctional potential ($V_j$) across the coupled cells. We analyzed the characteristics of this sensitivity and their effect on neuronal signaling, studying rectifying junctions present in the leech nervous system. The NS neurons, a pair of premotor nonspiking neurons present in each midbody ganglion, are electrically coupled to virtually every excitatory motor neuron. Studied at rest, only hyperpolarizing signals can be transmitted from NS to the motoneurons, and only depolarizing signals are conducted in the opposite direction. Our results show that small changes in the NS membrane potential ($V_m$) exerted an effective control of the firing frequency of the CV motoneurons (excitor of circular muscles). This effect revealed the existence of a threshold $V_j$ across which the electrical synapse shifts from a nonconducting to a conducting state. The junction can operate as a relatively symmetrical bidirectional bridge provided that the transmitted signals do not cross this threshold transjunctional potential.

**INTRODUCTION**

Electrical synapses have been shown to play significant roles in neural processing in the central nervous systems of invertebrate and vertebrate adults (Auerbach and Bennett 1969; Edwards et al. 1999; Furshpan and Potter 1959; Galarreta and Hestrin 2001; Llinás et al. 1974; Nicholls and Purves 1970; Veruki and Hartveit 2002). The electrical properties of gap junctions, the intercellular channels underlying this form of cellular communication (Kumar and Gilula 1996), determine the characteristics of signal transmission. The common view of electrical synapses as plain bidirectional channels between cells represents only a partial picture of their communication capabilities (Bennett 1997; Hormuzdi et al. 2004; Rela and Szczupak 2004). Interestingly, the initial descriptions of electrical coupling in the nervous system were made on rectifying junctions in the giant synapse in the crayfish (Furshpan and Potter 1959) and in the hatchet fish (Auerbach and Bennett 1969) where the junctions allow the transmission of excitatory signals from premotor interneurons to the motoneurons and inhibitory signals in the opposite direction.

The conductance of gap junctions displays two types of voltage sensitivities: most of them are sensitive to the transjunctional potential ($V_j$, the difference in potential across the gap) (Barrio et al. 1991; Jaslove and Brink 1986; Spray et al. 1979; Oh et al. 1999; Verselis et al. 1994), but they can also be sensitive to the transmembrane potential ($V_m$) of either cell (Obaid et al. 1983; Revilla et al., 2000; Verselis et al. 1991). Sensitivity to $V_j$ can be symmetrical when the junctional conductance is affected similarly by either polarity (Barrio et al. 1991; Landesman et al. 1999; Moreno et al. 1991; Spray et al. 1979), but it can also be asymmetrical when the junctional conductance is affected by the polarity of the transjunctional potential (Barrio et al. 1991; Bukauskas et al. 1995; Dykes et al. 2004; Jaslove and Brink 1986; Verselis et al. 1991). Although most studies have been performed in expression systems, or in dissociated cells, information on the voltage dependence of electrical synapses studied in their in situ configuration is scarce.

The present study investigates the voltage dependence of rectifying junctions present in the nervous system of the leech. The NS neurons (located in position 151 in the ganglion map) are a pair of premotor nonspiking neurons, present in each midbody ganglion of the leech CNS that extend profuse arborization. These nonspiking neurons are electrically coupled to virtually every excitatory motor neuron in the ganglion (Wadepuhl 1989). Studied at their normal resting potential, only hyperpolarizing signals can be transmitted from NS to the motoneurons, and only depolarizing signals are transmitted in the opposite direction. In terms of the motoneurons, the rectifying synapses described here conduct depolarizing signals in the opposite direction than those of the crayfish and the hatchet fish because in the latter, the rectification mediates excitation of the motoneurons, whereas in the leech, it mediates their inhibition. We had previously shown that the excitation of leech motoneurons produce inhibitory postsynaptic potentials in the NS neurons that, in turn, can be transmitted back to the motoneurons through the rectifying electrical synapses and regulate motor activity (Rela and Szczupak 2003). Therefore an analysis of these rectifying electrical junctions becomes relevant for the understanding of motor tuning in the leech nervous system.

Our results indicate that this rectification derived from the $V_j$ sensitivity of the electrical synapses. The interaction between the neurons discloses a threshold $V_j$ across which the electrical synapse shifts from a nonconducting to a conducting state. Experimental results show that within the conducting state the synapse can transmit depolarizing or hyperpolarizing signals.

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METHODS

Biological preparation

_Hirudo medicinalis_, weighing 2–5 g, were obtained from a commercial supplier (Leeches USA, Westbury, NY) and maintained at 15°C in artificial pond water. The animals were not fed for ≥1 mo prior to dissection. Individual ganglia were dissected out of the animal and pinned to silicone elastomer (Sylgard; Dow Corning) in a superfusion chamber at room temperature (around 20°C). The sheath covering the ganglion was dissected away, leaving the neuronal cell bodies exposed to the saline solution (containing, in mM, 115 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 5.4 Tris base, and 10 glucose; pH 7.4). To block synaptic transmission, we used a solution with a high-Mg²⁺/Ca²⁺ ratio (7 mM MgCl₂ and 1 mM CaCl₂) (Calviño et al. 2005). In this solution, the osmolarity was kept constant by reducing the NaCl concentration. The bathing solutions were applied through the perfusion system.

Electrophysiological recordings

Neuronal activity was recorded using intracellular glass microelectrodes connected to an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) operating in the bridge configuration. Microelectrodes were pulled from borosilicate capillary tubing (FHC, Brunswick, ME) and filled with a 3 M potassium acetate solution. Electrodes with a resistance of 40–60 MΩ were selected. The recordings were digitized using a Digidata 1320 interface and acquired using Clampex protocols (pClamp 9.2, Axon Instruments) at sampling frequencies of 5–10 kHz. The neurons were identified by their location, size, electrophysiological properties (Müller et al. 1981), and synaptic connections (Müller et al. 1981). Where stated, the membrane potential of the neurons was shifted to different values by injecting DC current through the bridge balanced recording electrode. The recording electrode was also used to inject current stimuli into the neurons.

Histological procedures

Identified neurons were impaled with an intracellular electrode the tip of which was filled with Lucifer yellow (10% dissolved in 100 mM LiCl) or rhodamine dextran 3K (10% dissolved in distilled water). Lucifer yellow and rhodamine dextran (Molecular Probes) were injected into the cells by iontophoresis, using 4-nA pulses (500 ms, 1 Hz) with negative polarity to deliver Lucifer yellow and positive polarity to deliver the dextran. The ganglia were fixed with 4% paraformaldehyde and then dehydrated, cleared in methyl salicylate, and mounted in a mounting medium (DePex; Serva).

The fluorescent images were examined using either a 488-nm Argon laser line or a 543-nm HeNe laser line of an Olympus FLUOVIEW FV300 confocal microscope.

Data analysis

The recordings were analyzed using commercial software (Axograph 4.5, Axon Instruments). Curve fitting was achieved using commercial software (Kaleidagraph 4.0, Abelbeck Software).

The basal membrane potential of the neurons, as indicated in each figure, was measured as the average potential of the trace (Figs. 2 and 3) or the average potential before applying the current pulse (Figs. 1, 4, and 5). Because the attenuated action potentials of the CV motoneurons were only a few millivolts in amplitude at the soma and because these are short-duration events, they did not alter significantly the actual baseline V_m = V_0 (this was confirmed by low-pass filtering the recordings at 2 Hz to eliminate the spikes).

In Figs. 2 and 3, the firing frequency was calculated by dividing the number of action potentials over the trace duration. In Fig. 4, the relative firing frequency was calculated as \( f_o / f_o' \), where \( f_o \) is the firing frequency of the CV motoneuron during the stimulus and \( f_o' \) is the firing frequency of the CV motoneuron for an equivalent period before the stimulus. In Figs. 2B and 4B, the values of \( V_m \) were binned every 10 mV, and the values of firing frequency in each bin were averaged.

FIG. 1. The NS neurons and most motoneurons are electrically coupled via rectifying electrical synapses. A: scheme represents the connections between the bilateral pair of NS neurons (in black) and the excitatory motoneurons CV, AE, cell 3 and L (in gray) within a single ganglion. The NS cells are coupled to each other through nonrectifying electrical junctions and to the motoneurons through rectifying electrical junctions. CV, excitator of the ventral longitudinal muscles; AE, annulus erecter, excitator of the annuli muscles; 3, excitor of the dorsal longitudinal muscles; and L, excitor of the dorsal and ventral longitudinal muscles. B: confocal micrograph showing the pair of NS neurons and 1 of the 2 CV motoneurons in a midbody ganglion (anterior up). The image results from a projection stack of optical sections (41 µm were scanned at 0.8-µm sections), using sequential, single laser line scanning for each fluorophore throughout the entire z axis. C: confocal micrograph showing only the morphology of the CV motoneuron (red channel in B). D: confocal micrograph showing only the morphology of the NS neuron (green channel in B). E: representative paired recordings of an NS neuron and a CV motoneuron when square current pulses, of positive and negative polarity, were applied to the former (right) or to the latter (left). The square lines indicate the timing and polarity of the current pulses and are located immediately above or below the recordings of the injected neuron. The experiments were performed in a solution containing 7 mM Mg²⁺ and 1 mM Ca²⁺.

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The results are expressed as average value ± SE and the number of neurons—or pairs of neurons—studied is expressed between brackets (n). Statistical analysis was performed with GraphPad Prism 4 for Windows (GraphPad Software). Nonparametric tests were performed owing to the small size of the samples (Sprent and Smeeton 2001). We used the Friedman test to analyze the differences in repeated measures (Fig. 2) and the Wilcoxon test to compare paired samples. All linear fits correspond to linear regression analyses and F tests were used to compare the slopes and elevations of the lines in Fig. 4.

RESULTS

In the present study, we have characterized the electrical synapse linking the nonspiking NS cells with motoneurons, focusing on the transmission of signals from the nonspiking cells to the CV motoneurons (Fig. 1A). These motoneurons innervate the ventral circular muscles and, as most motoneurons in the leech, are present as bilateral pairs in each midbody ganglion, innervating the contralateral side. Figure 1B presents superimposed confocal micrographs showing a pair of NS neurons and one of the two CV motoneurons in a midbody ganglion. Figure 1C shows the confocal micrograph of the CV motoneuron illustrating that it projects a neurite to the periphery through the contralateral anterior root and extends ipsilateral and contralateral arborizations within the ganglion. Figure 1D shows the confocal micrograph of the pair of NS neurons. This image resulted from filling one NS with Lucifer yellow, a dye that crosses only few electrical junctions in the leech nervous system. The pair of NS neurons is strongly electrically coupled, and both neurons show highly similar and highly correlated postsynaptic responses (Rela and Szczupak 2003). As it can be observed in the figure, NS neurons project extensive arborizations throughout the entire ganglion, and also through the peripheral roots and through the connective nerves projecting to adjacent ganglia. NS neurons are linked to CV motoneurons, as with virtually every excitatory motoneuron (Wadepuhl 1989), through rectifying electrical junctions. Analysis of the different optical sections (0.8 μm wide) of this confocal image shows several regions where the neurites of

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Influence of the NS membrane potential on the firing frequency of motoneurons. A: representative recordings of a CV motoneuron, recorded while an NS neuron in the ganglion (in normal saline solution) was set at different membrane potentials, indicated on the gray bar at the left. The baseline $V_{mCV}$ is indicated underneath each trace. The CV spikes recorded at the soma are of small amplitude due to the passive invasion of fully developed action potentials initiated at an electrically distant site. B: graphs show the relative firing frequency of CV motoneurons, and the change in $V_{mCV}$ ($\Delta V_{mCV}$), produced by $V_{mNS}$ manipulation. The CV firing frequency was made relative to that measured when $V_{mNS}$ was at +10 mV (in this condition the average CV firing frequency was 6.9 ± 0.5 Hz). The line indicates the linear regression calculated for the data points between $V_{mNS}$ ~90 and ~50 mV ($r^2 = 0.98$). Statistical analysis showed that both the CV firing frequency and the $\Delta V_{mCV}$ significantly changed with $V_{mNS}$ ($P < 0.01$, Friedman test). The symbols and error bars indicate mean ± SE, respectively (n = 5 pairs of cells).

The results are expressed as average value ± SE and the number of neurons—or pairs of neurons—studied is expressed between brackets (n). Statistical analysis was performed with GraphPad Prism 4 for Windows (GraphPad Software). Nonparametric tests were performed owing to the small size of the samples (Sprent and Smeeton 2001). We used the Friedman test to analyze the differences in repeated measures (Fig. 2) and the Wilcoxon test to compare paired samples. All linear fits correspond to linear regression analyses and F tests were used to compare the slopes and elevations of the lines in Fig. 4.

**FIG. 3.** Relationship between $V_{mCV}$ and the firing frequency of the motoneuron. A: representative recordings of a CV motoneuron as current injection into its soma shifted its $V_{m}$. The CV motoneuron, set initially at around ~20 mV, was subjected to a series of hyperpolarizing pulses (1 s). The figure presents a few of these traces, showing only the responses throughout the pulse. The numbers below each trace indicate $V_{mCV}$. B: relationship between firing frequency and $V_{mCV}$ for 6 different CV motoneurons. The lines correspond to linear regressions ($r^2$ between 0.9 and 0.98), with slopes ranging between 0.85 and 1.13 Hz/mV.
both neurons come into intimate juxtaposition. When both neurons are studied at their resting potential, in a solution containing 7 mM Mg$^{2+}$ and 1 mM Ca$^{2+}$ to suppress chemical interactions (see METHODS), only depolarizing signals are transmitted from CV to NS and hyperpolarizing signals from NS to CV (Fig. 1E). Similar results were observed in another two pairs of cells. However, under normal conditions, excitation of motoneurons activates, in addition, a chemical polysynaptic pathway that causes the hyperpolarization of NS and normally overrides the positive signal transmitted by the rectifying electrical junctions (Rela and Szczupak 2003). Therefore these rectifying electrical junctions are more effective in transmitting hyperpolarizing signals from NS to motoneurons than depolarizing signals in the opposite direction and thus this study focused mostly on the former.

NS neurons regulate the firing frequency of CV motoneurons

In previous studies, the effect of NS on motoneuron activity was revealed by injecting large current pulses in NS neurons (Isla et al. 1999; Wadepuhl 1989). To evaluate the physiological effectiveness of NS membrane potential ($V_{mNS}$) on motor neuron activity, we have analyzed the effects of a more restricted range of $V_{mNS}$ on the firing frequency of CV motoneurons. For this purpose, the CV motoneurons were initially set at a membrane potential depolarized from rest to establish a basal firing level. Figure 2A displays representative recordings of a CV motoneuron in normal solution, while an NS neuron was set at different membrane potentials (–90 to –10 mV), showing that the firing frequency of the motoneuron decreased as $V_{mNS}$ was shifted to values more negative than rest (around –40 mV). Depolarization of NS, on the other hand, had no effect on CV firing. Figure 2B gives a quantitative summary ($n = 5$ pairs of cells) of these results, revealing that the relationship between CV firing frequency and $V_{mNS}$ had a turning point at around –50 mV, suggesting a shift from an ohmic link to an uncoupled state. The average CV firing frequency and $V_{mCV}$ when $V_{mNS}$ was at –40 mV were 7.5 ± 0.85 Hz and –28 ± 1.4 mV, respectively. It is noteworthy that
changes in the CV firing frequency were achieved with very little change in the baseline membrane potential (Fig. 2B). One should take in consideration the information provided by Fig. 1 showing that the somata of NS and CV are spatially segregated, that NS cells extend a very broad arborization and the electrical junctions with CV take place at sites that are probably electrically distant from the soma.

The results presented in Fig. 2 show that changes in $V_{mNS}$ affect the activity of the CV motoneuron. The most straightforward interpretation of this observation is that the changes in $V_{mCV}$ that we recorded at the soma reflect changes in $V_{mCV}$ at the spike initiation zone and, in turn, this affects the firing frequency of the motoneuron. For this interpretation to be correct, we should find a linear relationship between the somatic $V_{mCV}$ and the CV firing frequency. To test this, we analyzed how direct manipulation of $V_{mCV}$ affected CV activity. Figure 3A shows a representative example of a CV motoneuron the membrane potential of which was manipulated by DC injection into its soma, and B shows a quantitative analysis performed for six different CV neurons. It is possible to observe that the firing frequency was a linear function of the somatic $V_{mCV}$ with an average slope of $1.04 \pm 0.11$ Hz/mV.

**Determination of the threshold for activation of the electrical synapse**

The experiments presented so far showed that the electrical coupling between NS and CV neurons enabled a linear relationship between $V_{mNS}$ and CV firing frequency that manifested an activation threshold that probably depended on the junctional potential. To test this hypothesis, we performed a series of experiments shifting both $V_{mNS}$ and $V_{mCV}$. In the implementation of these experiments, one has to take into consideration that changes in $V_m$ can cause changes in the electrical properties of the neurons (input resistance and, through it, length constant), thus affecting the electrotonic spread to and from the electrical junction. Because NS is the presynaptic cell in our experiments, it is less likely that changes in its electrophysiological properties have an impact on measurements of the electrical coupling (Mann-Metzer and Yarom 1999). Instead changes in $V_{mCV}$ produce changes in the input resistance of this neuron that could cause, to a large extent, changes in coupling measurements (Rela and Szczupak 2003).

On this basis, here we have analyzed a broad range of $V_{NS-CV}$ (the $V_j$ of the electrical synapse calculated as $V_{mNS}-V_{mCV}$), manipulating mainly $V_{mNS}$. CV motoneurons were shifted between two discrete membrane potentials, $-30$ and $-10$ mV, at which the input resistance values were $40.7 \pm 4.0$ and $29.4 \pm 5.1 \text{ M\Omega}$ ($n = 5$ CV neurons, not statistically different as tested by the Wilcoxon test), respectively. At both potentials, CV fired steadily. Our strategy was to study the CV firing frequency as a function of $V_{mNS}$, expecting that this variable would give us a more sensitive measurement of the activation of the electrical synapse than the routinely used coupling coefficient because the spike frequency was a more sensitive variable than changes in $V_m$ (Fig. 2). Figure 4A shows representative recordings of the experiments performed: the NS neuron was set initially at 0 mV, and its membrane potential was shifted by applying square current pulses of increasingly negative amplitude, while recording a CV motoneuron set at $-30$ (left) and at $-10$ mV (right). If the coupling depended mainly on $V_{mNS-CV}$, we predict that the $V_{mNS}$ value causing the first detectable change in CV firing should be more positive for the series tested with $V_{mCV}$ at $-10$ mV than for the one tested at $-30$ mV. The firing frequency was considered to be different from basal level when the average firing frequency during the pulse was, at least, 10% smaller than the firing frequency in an equivalent period preceding it. The results show that shifting $V_{mNS}$ caused no detectable changes in CV firing frequency up to certain amplitude of the stimulus (indicated by the arrows in Fig. 4A); further negative shifts in $V_{mNS}$ produced progressively larger changes in CV firing frequency. In this context, we will call this $V_{mNS}$ level the threshold $V_{mNS}$. The resulting picture is very similar in the two conditions tested, namely CV motoneuron at $-30$ mV or at $-10$ mV, except that the threshold $V_{mNS}$ had a more positive value as CV was held at a more positive potential. Figure 4B gives a quantitative summary of the results ($n = 7$ pairs of cells) obtained in these series of experiments expressed as the relative firing frequency of CV as a function of $V_{mNS}$. The average firing frequency values before the pulse were $18.8 \pm 5.3$ and $31.6 \pm 3.0$ Hz for CV at $-30$ and at $-10$ mV.
respectively. The data indicate that the firing frequency of CV neurons, set at −10 or at −30 mV, varied linearly with \( V_{\text{mNS}} \), but the points corresponding to CV set at −10 mV lined to the right (by 19 ± 9 mV) of those corresponding to CV set at −30 mV. In other words, to produce an equivalent reduction in the firing frequency of the CV neurons \( V_{\text{mNS}} \) had to be further hyperpolarized the more hyperpolarized the CV neuron was. It is noteworthy that the CV input resistance tended to be smaller at −10 mV than at −30 mV (see preceding text), but yet the motoneurons were able to report an NS deflection of smaller amplitude. Figure 4Bi illustrates the data from the two series of experiments as a function of the transjunctional potential \( V_{\text{NS-CV}} \), evidencing an ample superposition of the two series of points.

These results support the hypothesis that the activation threshold of the NS-CV synapse depended on the transjunctional potential. Extrapolation of the linear fits in Fig. 4Bi to a relative firing frequency of 1 (no change) indicates the threshold \( V_{\text{mNS}} \) were 17 ± 3 and 15 ± 3 mV for CV at −10 and at −30 mV, respectively. This suggests that the threshold \( V_{\text{NS-CV}} \) was of around 16 mV and that the electrical synapse persisted in a conducting state for values more negative than it. However, because these are somatic recordings, the actual threshold \( V_{\text{NS-CV}} \) at the gap junction site cannot be determined on the basis of these experiments.

Electrical synapse between NS and CV neurons allow the passage of depolarizing and hyperpolarizing signals in both directions, depending on \( V_{\text{NS-CV}} \)

The recordings shown in Fig. 1 were obtained while both cells were held at a similar \( V_{\text{m}} \) thus \( V_{\text{NS-CV}} \) was close to zero. According to the results shown in Fig. 4 this \( V_{\text{NS-CV}} \) value is more negative than the activation threshold (16 mV), and therefore the junction was expected to be in its conducting state. In this condition, depolarizing pulses injected in CV were transmitted to NS but hyperpolarizing ones were not. Analyzed under the concept of an activation \( V_{\text{NS-CV}} \) threshold, the results from Fig. 1 can be explained as follows: depolarization of CV was transmitted to NS because this change in \( V_{\text{mCV}} \) drives \( V_{\text{NS-CV}} \) to potentials at which the electrical junctions remain in their conducting state; instead, hyperpolarization of CV was not transmitted because it drives \( V_{\text{NS-CV}} \) to potentials at which the junctions are not conducting.

If this interpretation is correct, one could manipulate \( V_{\text{mCV}} \) and \( V_{\text{mNS}} \) in such a way that hyperpolarization of CV could render a hyperpolarizing response in NS provided that this hyperpolarizing signal would not drive \( V_{\text{NS-CV}} \) to values more positive than threshold. Figure 5A shows a representative example of such a condition: setting CV at an initial potential of around −20 and NS at around −70 mV provided for a condition where hyperpolarization of the motoneuron could be transmitted to the NS neuron. Instead if CV was at a membrane potential close to \( V_{\text{mNS}} \), the signal was not transmitted (and also see Fig. 1). The conductance state of the electrical junctions did not depend on the absolute value of \( V_{\text{mNS}} \) but on satisfying the condition that the change in \( V_{\text{mCV}} \) did not drive \( V_{\text{NS-CV}} \) to values more positive than threshold. Similarly, depolarization of NS could be transmitted to CV when the latter was set at a potential more positive than NS, and the pulse did not bring \( V_{\text{NS-CV}} \) to values more positive than threshold (Fig. 5B). Instead, when \( V_{\text{mNS}} \) was set at a value that shifted \( V_{\text{NS-CV}} \) to values more positive than threshold, the electrical synapse was in a nonconducting state. Other two pairs of cells showed similar responses.

**Discussion**

Excitation of motoneurons in the leech produce inhibitory potentials in NS neurons (Rela and Szczupak 2003; Wadepuhl 1989) that, in turn, can be transmitted back to motoneurons through the rectifying electrical synapses linking NS to virtually every excitatory motoneuron. We had previously shown that this loop circuit could play a role in adjusting motor activity (Rela and Szczupak 2003), and here we have focused our attention on the interaction between NS and CV motoneurons on the assumption that it is representative of the link between NS and most motoneurons.

**Efficacy of the rectifying synapse between NS and motoneurons in the control of motor activity**

Our results show that hyperpolarization of NS neurons to −60 mV can cause a decrease of ~20% in CV firing frequency (Fig. 2B). Considering that we manipulated \( V_{\text{mNS}} \) at the soma, it is likely that our experimental data underestimate the efficiency of the interaction between NS and CV. Figure 1B illustrates that the electrical coupling between these neurons must occur at sites electrically distant from the soma and therefore potential changes imposed at the soma may suffer substantial attenuation as they reach the junction site, and even more so for dynamic \( V_{\text{m}} \) changes due to AC filtering.

Given that NS neurons exert similar actions on virtually every excitatory motoneuron in the leech (Wadepuhl 1989), we believe that the results described here can be generalized—despite quantitative differences—to the interaction with most excitatory motoneurons. We thus conclude that relatively small changes in \( V_{\text{mNS}} \) could cause substantial changes in the electrophysiological activity of the whole population of excitatory motoneurons in the leech ganglion.

**Properties of the rectifying junctions linking the NS neurons and the motoneurons**

The analysis of electrical synapses in situ presents severe technical difficulties due to the fact that these synapses have a very local effect in the neuritic arbor far from where recordings are usually made, the soma. This is why the majority of biophysical characterizations of electrical junctions have been performed using expression systems. To circumvent part of the difficulties in the analysis of electrical junctions between NS and the CV motoneuron at their natural network configuration, we measured their function by analyzing the influence on the firing frequency of the postsynaptic cell. This variable can be used as a faithful sensor of changes in \( V_{\text{m}} \) taking place in the arborization of the neurons because spike initiation zones and gap junctions are likely to be closer together than either of these are to the soma.

Through such an analysis we could determine that the electrical junctions were activated at a \( V_{\text{NS-CV}} \) of −16 mV: when \( V_{\text{mCV}} \) was ≥16 mV more positive than \( V_{\text{mNS}} \) a linear interaction was found between \( V_{\text{mNS}} \) and the firing frequency of the CV motoneuron (Fig. 4). This linear fit suggests that
once threshold to open the connection was reached, the coupling strength was fairly constant. The concept of a threshold transjunctional potential in relationship to rectifying junctions becomes a key analytical element. It has also been applied in relationship to the coupling of amphibian blastomeres (Harris et al. 1983) and the crayfish giant synapse (Heitler et al. 1991).

The rectifying electrical synapses operate as asymmetrical junctions when $V_j$ is close to the activation threshold because the transmitted signals can readily shift this variable to values more positive than threshold and bring the electrical junctions into a non conducting state. However, they can also operate as relatively symmetrical bidirectional junctions depending on the initial $V_j$ (Fig. 5). The condition for a symmetrical transmission is that the transmitted signals do not cross the threshold $V_j$. Similar results were observed in the crayfish giant motor synapse (Giaume and Korn 1983).

It is accepted that in invertebrates gap junctions are constituted by protein molecules of the innexin family. In the leech, 12 different innexin genes have been cloned and their products are thought to mediate electrical coupling between leech neurons (Dykes and Macagno 2005). The majority of the neurons expresses the same innexin, inx1, but few neurons express in addition another two innexins, inx6 and inx8. Electrical junctions are not uncommon in the leech (Baylor and Nicholls 1969; Fernandez de Miguel et al. 2001); they are highly expressed during development, setting the ground for the formation of chemical synapses in the adult (Martin Burgin et al. 2005, 2006). In adults, most of the electrical junctions are formed between bilateral homologue cells that are linked by nonrectifying junctions, a fact consistent with homotypic gap junctions (formed by the same hemichannels). One exception to this rule is the electrical coupling between T sensory neurons, where doubly rectifying junctions have been described (Acklin 1988). Because heterotypic gap junction channels (formed by different hemichannels) have been shown to underlie asymmetric rectifying junctions (Barrio et al. 1991), it is likely that the NS-motoneuron junctions are formed by different hemichannels although the identity of the innexins involved remains to be established.

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