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Facilitation of antagonist motor output through short-latency sensory pathways during postnatal development in the mouse

Patrick M. Sonner^a and David R. Ladle^a

^aDepartment of Neuroscience, Cell Biology, and Physiology, Wright State University, Dayton, Ohio, USA

Abstract

Reciprocal inhibition of motor neurons via Ia inhibitory interneurons recruited by stimulation of proprioceptive afferents supplying antagonist muscles has been well described. Changes in the efficacy of inhibition, and sometimes even a switch from inhibition to facilitation, have been reported in the literature after disruption of descending pathways. We sought to test whether such facilitation could be expressed in normal animals by evaluating the presence of facilitation in acute preparations from uninjured animals. Using an isolated spinal cord preparation from neonatal mice, changes in the monosynaptic stretch reflex response in knee flexor motor neurons (posterior biceps semitendinosus; PBST) were monitored following conditioning stimulation of proprioceptive sensory afferents in other muscle nerves. As expected for reciprocal inhibition, conditioning by stimulation of quadriceps (knee extensors and PBST antagonists) sensory afferents resulted in inhibition of the stretch reflex response. Facilitation, however, of the stretch reflex response by quadriceps conditioning stimulation was observed when the glycinergic reciprocal inhibitory pathway was blocked by application of strychnine. Facilitation was elicited by low-threshold proprioceptive afferents and occurred at latencies consistent with a disynaptic circuit. The magnitude of facilitation was larger at birth than at one week postnatal. Our results also suggest reciprocal facilitation is restricted to antagonist muscle pairs, as facilitation of PBST responses was not observed when conditioned with the obturator nerve supplying the adductor muscles. Overall, these data suggest the efficacy of facilitation is modulated during the first postnatal week, while the specificity of facilitation is already established by birth.

Keywords

antagonists; co-contraction; development; spinal cord

Corresponding Author's Name, Address, and Email: D.R. Ladle, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435, (david.ladle@wright.edu), Fax: 937-775-3391.

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1. Introduction

Sensory feedback from muscle proprioceptors is necessary to update movement programs in response to changes in the physical environment [1]. This feedback includes excitation of target motor neurons (MNs) by direct, monosynaptic excitation, via the stretch reflex circuit. Other rapid feedback pathways can lead to excitation or inhibition of MNs via spinal circuits requiring only one (disynaptic) or two (trisynaptic) interneuronal relays in the spinal cord. Modulation of motor output by these pathways has been a focus of motor control neuroscience for decades and some pathways have been well characterized [2].

One such sensory feedback circuit mediates reciprocal inhibition [3]. This disynaptic inhibitory pathway utilizes a single class of glycinergic interneurons that receive monosynaptic sensory input from group Ia muscle spindle afferents and act to directly inhibit MNs projecting to muscles with antagonistic actions. A classic example of this circuit is the inhibition of knee flexor MNs (posterior biceps) following excitation of knee extensor (quadriceps) Ia afferents [4,5]. Ia inhibitory interneurons receive input from descending pathways and other spinal circuits, placing them in a key position to influence motor output [6].

The status of reciprocal inhibition can influence joint stiffness through regulation of muscle tone of the antagonist muscles at the joint, and the status of reciprocal inhibition has been explored in multiple diseases where motor coordination is compromised by spasticity, or altered joint stiffness [7–9]. Reported changes, however, have been variable. For example, among patient populations suffering from cerebral palsy with spasticity, some reports concluded reciprocal inhibition is strengthened [10], while in others it is unchanged [11], or even replaced by facilitation [12].

Examples of facilitation are particularly intriguing, as the reflex relationship of antagonist muscles demonstrates a reversed sign, promoting excitation compared to normal reciprocal inhibition. If facilitation is strong enough, co-contraction of antagonist muscle groups can result from activation of extensor spindle afferents. While simultaneous contraction of antagonist muscles at a joint is necessary in some motor tasks, co-contraction impedes many important motor functions, particularly when under normal circumstances the same sensory signals result in inhibition of antagonists.

Expression of facilitation may result from circuit plasticity in the spinal cord following injury to descending pathways [13]. Alternatively, circuits supporting facilitation could be present in normal spinal cords and distinct from those producing reciprocal inhibition. Indeed, activation of Ib Golgi tendon organ afferents evokes facilitation of antagonist MNs via a trisynaptic circuit [14]. In a previous publication using an *ex vivo* spinal cord preparation from wild-type neonatal mice, we noted facilitation of MN responses by stimulation of antagonistic sensory afferents when reciprocal inhibition was blocked pharmacologically [15]. In the current study, we investigate this phenomenon more thoroughly and demonstrate the existence of short-latency pathways in neonatal animals for sensory-evoked facilitation of antagonist MNs.

2. Materials and Methods

All animal procedures were approved by the Wright State University Animal Care and Use Committee. Two groups of neonatal mice (C57BL/6J) were used in this study: animals less than one day old (postnatal day P0/P1; n=16) and animals one week postnatal (P7/P8; n=27). Mice were anesthetized by hypothermia in an ice water bath and then transcardially perfused with artificial cerebral spinal fluid (ACSF) as previously described [15]. Isolated, hemisected spinal cords dissected in continuity with peripheral nerves supplying knee flexors (posterior biceps and semitendinosus; PBST) and extensors (quadriceps; Quad), as well as the adductor muscles (obturator, Obt) were prepared as described previously [5,15].

The parameters and equipment used for extracellular recordings of motor axon responses in the PBST nerve were described in a previous publication ([15]; see Fig. 1A for diagram of preparation). Stimulation of DRL5 activates the majority of Ia sensory afferents that supply the PBST and produces a large compound action potential (CAP) in the PBST peripheral nerve as a result of monosynaptic connections with PBST MNs. Test trials (T; DRL5 stimulation only) were interleaved every 10 seconds with conditioning stimuli (C; Quad or Obt) that preceded DRL5 stimulation (C+T trials). Intervals ranged from 0ms to 50ms (1 or 2ms increments). The L2 to L4 ventral roots were cut to prevent antidromic stimulation of Quad or Obt motor axons [5]. All trials were presented six times and the responses were averaged offline using custom routines in MATLAB (The MathWorks, Natick, MA). For each trace, the signal was rectified and integrated from the initial onset to the negative peak of the CAP in the T trial (Fig. 1B). The response ratio was calculated as C+T CAP area divided by T CAP area [15]. To block reciprocal inhibition, strychnine (0.4 μ M; Sigma-Aldrich, St. Louis, MO) was chosen due to its specificity for glycinergic receptors as described previously [5]. Data is presented as mean $\pm$ standard error of the mean, unless otherwise indicated. Statistical comparisons were performed using the nonparametric Wilcoxon rank sum test and Student's t-test. Results were considered significant if $P \leq 0.05$.

3. Results

We used an *ex vivo* spinal cord preparation isolated from neonatal mice to study sensory-motor circuits in the spinal cord that mediate interactions between antagonist muscle groups. Decreased motor responses in knee flexors (PBST) were observed following stimulation of proprioceptive afferents projecting to knee extensors (Quad) as described for classic reciprocal inhibition [4,5,15].

Application of strychnine (0.4 μ M) blocks the inhibition between Quad and PBST observed in our preparation, consistent with reciprocal inhibition being mediated by glycinergic interneurons [5,15]. Response ratios larger than one, however, were observed for similar conditioning intervals, suggesting Quad afferent activation in fact increases PBST MN activation, leading to facilitation in place of inhibition (Fig. 1D; P7/P8: $27.9 \pm 13.4\%$ facilitation, n=5; data from [15]). This phenomenon, present with even greater magnitude, was also observed at birth (Fig. 1C; P0/P1: $66.0 \pm 9.9\%$ facilitation, n=3; $P < 0.0005$, Wilcoxon rank sum test).

In the present study, we sought to characterize this phenomenon more completely. A simple explanation for apparent facilitation could be a removal of tonic glycinergic inhibition on MNs by application of strychnine. No significant change, however, was detected in the PBST CAP peak amplitude after strychnine application in either age group, suggesting MN activation is not limited by tonic glycinergic inhibition in normal ACSF (Fig. 1E; P0/P1 Control = 0.262 ± 0.075 mV; P0/P1 Strychnine = 0.234 ± 0.072 mV, $n=5$; $P = 0.296$, paired t-test; P7/P8 Control = 1.278 ± 0.416 mV; P7/P8 Strychnine = 1.368 ± 0.406 mV, $n=6$; $P = 0.102$, paired t-test). At birth, greater trial-to-trial variability was observed in the magnitude of facilitation than at one week postnatal, suggesting circuits mediating facilitation may be immature at this stage (Fig. 1F; Variance: P0/P1 = 2346.92 ± 844.86 , $n=5$; P7/P8 = 232.41 ± 77.94 , $n=11$; $P < 0.005$, unpaired t-test).

The onset latency of inhibition and facilitation was then determined using 1ms conditioning interval steps. On average, the onset latency for inhibition and facilitation differed by less than two milliseconds (inhibition onset 1.60 ± 0.24 ms; facilitation onset 3.40 ± 0.51 ms; P7/P8; $n=5$). While the onset latency was longer at P0/P1, due to immature myelination of peripheral nerves, average latencies were again similar for both inhibition and facilitation (inhibition onset: 13.40 ± 1.25 ms; facilitation onset 13.20 ± 1.02 ms; $n=5$). These results indicate sensory-evoked inhibition and facilitation are produced by spinal circuits with similar numbers of synaptic relays.

We next sought to determine if facilitation provided by Quad afferent pathways in the presence of strychnine was sufficient to drive PBST MN firing in the absence of cooperative DRL5 afferent stimulation. At birth (P0/P1) Quad nerve stimulation in normal ACSF elicited a series of CAPs in the PBST nerve, but with long onset latencies (Fig. 2A; 35.91 ± 3.43 ms; $n=9$). The onset latency in normal ACSF from one week old (P7/P8) animals was reduced (Fig. 2C; 23.20 ± 1.70 ms; $n=7$), but likely evoked by activation of polysynaptic, and not short-latency pathways [15].

In P7/P8 animals, $0.4\mu\text{M}$ strychnine resulted in a significant reduction in the latency to the first CAP (Fig. 2D; 16.41 ± 0.47 ms, $n=7$; $P=0.01$, two-tailed paired t-test). This corresponds closely to the predicted latency for a response mediated by a disynaptic spinal pathway in animals of this age when accounting for peripheral and central conduction delays (~ 15 ms; [15]). No change in onset latency was observed in P0/P1 animals following strychnine application (Fig. 2B; 36.95 ± 4.42 ms; $n=9$; $P=0.45$, two-tailed paired t-test).

We sought to determine the general class of afferents responsible for facilitation using stimulation intensities just above threshold, to eliminate possible contributions from higher threshold, non-proprioceptive afferents (groups III and IV) present in the Quad nerve. We found threshold stimulation ($1.0 \times T$), on average, elicited a modest degree of both reciprocal inhibition and facilitation, with maximal changes observed at $1.5 \times T$ (Fig. 3A, B). In four of eight preparations, inhibition and facilitation were evoked at the same intensity (Fig. 3C). In the other preparations, facilitation was evoked by slightly higher stimulus intensities. Facilitation was generally greater in preparations that had the same threshold for facilitation and inhibition, although results did not reach significance (Fig. 3D; Same Threshold = 1.66 ± 0.13 , $n=4$; Different Threshold = 1.29 ± 0.14 , $n=4$; $P=0.08$, Wilcoxon rank sum test). The

activation of facilitation by low-threshold ($<1.5 \times T$) afferents, suggested facilitation was mediated by proprioceptive afferents.

The adductor muscles do not have strict antagonistic or synergistic relationships with either the quadriceps or PBST muscle groups. We tested if facilitation of PBST MNs could be evoked by stimulation of adductor sensory afferents (found in the Obturator nerve). In these experiments, alternating trials using either the Quad or Obturator nerve as conditioning sources were interleaved with DRL5 test pulses. Conditioning stimulation using the Obturator nerve did not result in any change to the response ratio in either age or drug group (Figs. 4A–B; P0/P1: Control ACSF = 0.94 ± 0.02 ; Strychnine = 0.98 ± 0.02 ; $n=4$; $P=0.08$, two-tailed paired t-test; P7/P8: Control ACSF = 0.978 ± 0.010 ; Strychnine = 1.033 ± 0.025 ; $n=4$; $P=0.09$, two-tailed paired t-test). We also measured the onset latency of PBST CAPs evoked by stimulation of the Obturator nerve, in the absence of DRL5 stimulation (see Fig. 2A for comparison). Onset latencies in normal ACSF were similar to those observed after Quad nerve stimulation in both P0/P1 and P7/P8 animals (Fig. 4C–H; P0/P1: Control ACSF = 39.61 ± 8.60 ms; P7/P8: Control ACSF = 20.15 ± 1.01 ms). Unlike stimulation of Quad afferents, however, the onset latency in P7/P8 animals was not changed in the presence of strychnine (Fig. 4F–H; Strychnine = 21.53 ± 0.54 ms; $n=3$; $P=0.46$; two-tailed paired t-test).

4. Discussion

In this study we have characterized a sensory-evoked pathway that can lead to facilitation of antagonistic MNs in isolated spinal cord preparations from neonatal mice. Facilitation is only observed when reciprocal inhibitory pathways are blocked by application of strychnine. The acute nature of these experiments demonstrate functional neonatal spinal network connections and circuits exist to support this circuit behavior. This facilitation not likely to be explained by changes in intracellular chloride concentrations in MNs leading to depolarizing effects of glycine postulated by Boulenguez and colleagues [13], as facilitation is immediately observed and glycine receptors are blocked with strychnine. Facilitation is elicited through stimulation of low-threshold proprioceptive afferents, is observed at birth, and is sufficient by one week postnatal to drive PBST MN firing in the presence of strychnine. Finally, facilitation is evoked only by stimulation of afferents supplying antagonists and not by proprioceptive afferents from functionally unrelated muscles, suggesting specificity of action.

Facilitation, as opposed to reciprocal inhibition, of antagonists can be observed in humans with several clinical conditions. Children suffering with cerebral palsy, for example, maintain an abnormally high degree of co-contraction of antagonist muscles [16] and reduced reciprocal inhibition [17] making refined coordinated movements difficult. Concomitantly, reflexive “reciprocal excitation” between ankle antagonists has been noted in these patients [18]. Patients with the major form of hyperekplexia, a rare hereditary condition, have significantly reduced reciprocal inhibition [19]. In several of these patients, facilitation evoked by stimulation of antagonist afferents was observed at a latency in agreement with a disynaptic circuit [19].

Clinical reports of facilitation have employed a conditioning-plus-test approach, as in our study. While these studies agree facilitation is mediated via a short-latency pathway, not all observe facilitation with the same latencies, and by extension, the same synaptic relays. Crone, *et al.*, suggest facilitation begins at the same latency as reciprocal inhibition (an exclusively disynaptic pathway), thus inferring facilitation is disynaptic [20]. Others report latencies in between those expected for di- and tri-synaptic pathways, suggesting facilitation may contain both di- and trisynaptic elements [19,21,22].

Facilitation may be produced through several identified disynaptic or trisynaptic pathways between proprioceptive afferents and MNs, yet no single pathway adequately accounts for all aspects of our findings. Candidate disynaptic pathways must be activated by proprioceptive sensory input and act to excite MN targets that project to antagonist muscles. While disynaptic excitation of MNs has been observed in response to group I proprioceptive afferent stimulation during locomotion, the MN targets do not include antagonists [23]. Facilitation of antagonists by Ib afferent activation has been well documented, but this occurs via a trisynaptic pathway [14,24]. Conditioning stimuli in our neonatal preparation activates both muscle spindle and GTO afferents, therefore Ib facilitation likely contributes across the relatively broad time course of facilitation beginning at a trisynaptic latency. We observed, however, sensory-evoked facilitation and inhibition have similar onset and peak latencies, suggesting these opposing effects are mediated by spinal circuits containing the same number of synaptic relays in the spinal cord. Sensory-evoked reciprocal inhibition is a disynaptic pathway mediated by glycinergic Ia inhibitory interneurons [3]. The alignment of onset latencies between reciprocal inhibition and facilitation, suggests the presence of a disynaptic circuit capable of producing proprioceptive sensory neuron-evoked excitation of antagonists.

Interneurons responsible for disynaptic facilitation must receive direct input from proprioceptive afferents and make excitatory connections with ipsilateral MNs. Interneurons of the dI3 class are a potential candidate population. They receive both cutaneous and proprioceptive primary afferent input and project to ipsilateral MNs [25]. While cutaneous mechanoreceptor contributions to motor control via dI3 spinal pathways have been confirmed, potential proprioceptive modulations of MN activity via this pathway have not yet been investigated [25].

In summary, we have shown low-threshold proprioceptive afferents can recruit a spinal interneuron pathway leading to facilitation of MNs supplying antagonistic muscles. This pathway is present in normal animals and undergoes developmental changes during the first postnatal week. Future studies could identify interneurons responsible for this phenomenon and attempt to elucidate the activity and role of facilitation in normal conditions.

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Highlights

- Reciprocal inhibition classically leads to inhibition of antagonist motor neurons.
- Proprioceptive afferents can also evoke excitation in antagonist motor neurons.
- Facilitation occurs at latencies consistent with a disynaptic circuit.
- Facilitation is restricted to antagonist muscle pairs.

Fig. 1. Stimulation of muscle sensory afferents facilitates antagonist motor neuron activation following blockade of glycinergic transmission. **A:** Schematic diagram of preparation illustrating electrodes for conditioning stimulation (C) of Quad afferents, test pulse (T) stimulation of the L5 dorsal root (DRL5), and for recording PBST responses. **B:** Representative average traces of PBST nerve compound action potentials (CAP) from a P7 animal. Test pulse alone (black trace, T) shows monosynaptic activation of PBST motor neurons via stimulation of DRL5 afferents. After pharmacological blockade of glycinergic signaling (bath application of strychnine), a conditioning stimulus of Quad afferents enhanced the PBST response (C + T). Gray boxes indicate time interval used in analysis. **C–**

D: Response ratios obtained at various conditioning intervals from representative P0 (**C**) and P8 (**D**) preparations in normal ACSF (filled squares) and after addition of 0.4 μ M strychnine (open squares). Negative conditioning intervals indicate series where the test pulse preceded the conditioning pulse. A conditioning interval of 0ms indicates synchronous stimulation of Quad and DRL4 afferents. **E:** Average test (T) CAP peak amplitude measured before and after application of strychnine. **F:** Variance of the response ratios associated with maximal facilitation at birth (P0/P1) and one week postnatal (P7/P8). Error bars indicate standard deviation (**C, D**) or standard error of the mean (**E, F**).

Fig. 2. PBST motor neuron responses following Quad sensory afferent stimulation. **A–D:** Representative single traces showing PBST CAPs evoked by Quad nerve stimulation at birth (**A, B**) and at one week postnatal (**C, D**). Arrows point to the first CAP in each trace. **E, G:** Percent histograms from representative preparations at birth (**E, P1**) and one week postnatal (**G, P7**) of latency to the first CAP across 100 trials (at 0.1Hz) before and after application of strychnine. Gaussian functions were fit to both control (gray dashed line) and strychnine (solid black line) groups. **F, H:** Mean latency of first CAP for each isolated spinal cord preparation at birth (**F**) and one week postnatal (**H**). Lines connect data points from same

preparations before and after strychnine application. Mean and standard error are also shown in **F** and **H**.

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Fig. 3.

Reciprocal facilitation is activated by low-threshold afferents. **A:** Representative data from one preparation (P8) depicting response ratios characteristic of reciprocal inhibition and facilitation observed at various multiples of threshold intensity (1.0 – 1.5×T) before and after application of strychnine. **B:** Average maximum reciprocal inhibition or facilitation as a function of stimulation intensity at one week postnatal. **C:** Comparison of stimulation intensity required to elicit reciprocal inhibition and facilitation for each of eight P7/P8 preparations. In some preparations, facilitation was evoked at the same stimulus intensity as inhibition (blue lines), while in others greater stimulus intensity was required (yellow lines). Parentheses indicate the number of cases when multiple data points had the same stimulus intensity. **D:** Average maximal response ratio observed when reciprocal inhibition and facilitation were activated at the same stimulus intensities (n=4) compared with preparations in which different stimulus intensities were required (n=4). Error bars indicate standard deviation (**A**, **B**) or standard error of the mean (**D**).

Fig. 4.

Reciprocal facilitation is not evoked by conditioning stimulation of functionally unrelated muscle nerves. **A, B**: response ratios across varying conditioning latencies following conditioning stimulation of Quad (boxes) and Obturator (Obt) nerve (circles) before (filled) and after strychnine application (open). Representative data from single preparations at P0 (**A**) and P8 (**B**) are shown. **C–H**: Patterns of PBST activation following Obt nerve stimulation at birth (**C–E**) and one week postnatal (**F–H**). **C, F**: Latency to first PBST CAP following Obt stimulation both before and after strychnine application at birth ($n=4$; **C**) and one week postnatal ($n=3$; **F**). Representative traces of PBST CAPs from a single experiment (P1, **D, E**; P7, **G, H**) are shown. Error bars indicate standard deviation (**A, B**) or standard error of the mean (**C, F**).