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Research article

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

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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.

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

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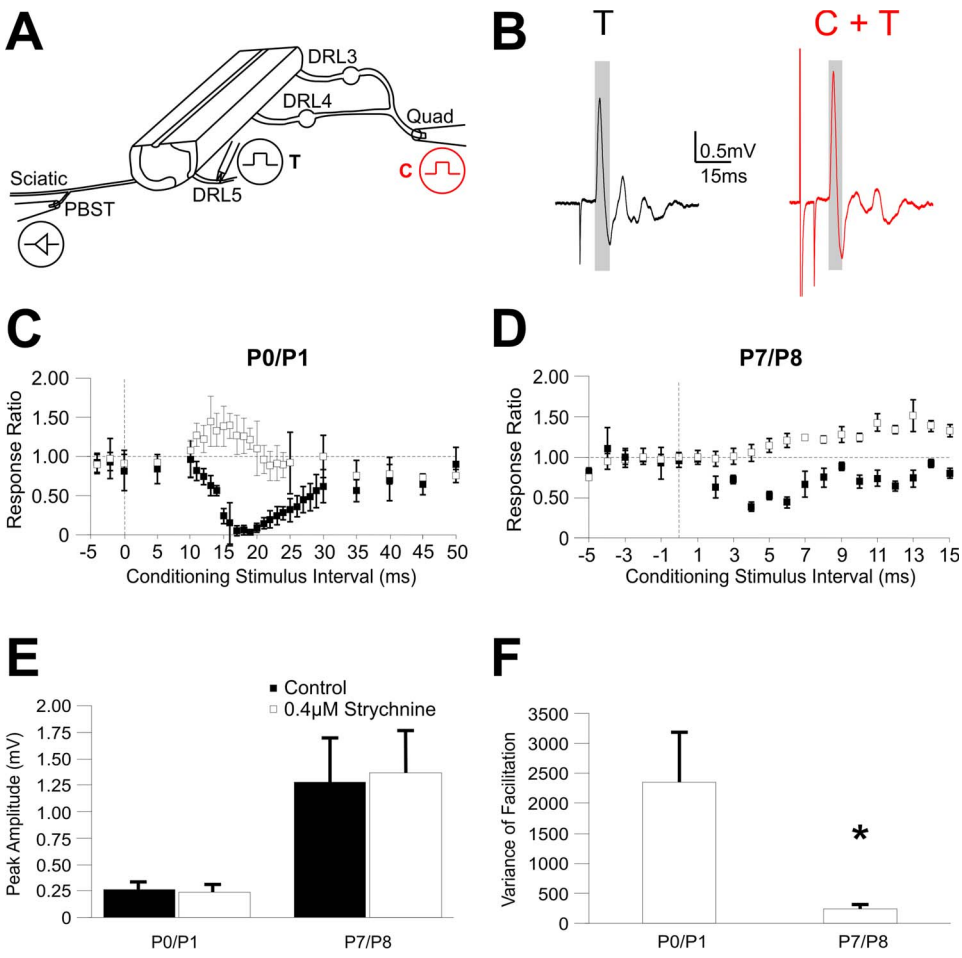

Fig. 1. Stimulation of muscle sensory afferents facilitates antagonist motor neuron activation following blockade of glycinergic transmission. A: Schematic diagram of preparation illustrating electrodes for conditioning stimulation (C) of Quad afferents, test pulse (T) stimulation of the L5 dorsal root (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 μM strychnine (open squares). Negative conditioning intervals indicate series where the test pulse preceded the conditioning pulse. A conditioning interval of 0 ms 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).

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 s with conditioning stimuli (C; Quad or Obt) that preceded DRL5 stimulation (C + T trials). Intervals ranged from 0 ms to 50 ms (1 or 2 ms 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 μM; Sigma-Aldrich, St. Louis, MO) was chosen due to its specificity for glycinergic receptors as described previously [5]. Data is presented as mean ± 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