Intracellular Analysis of Reflex Pathways Underlying the Stumbling Corrective Reaction During Fictive Locomotion in the Cat

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Intracellular analysis of reflex pathways underlying the stumbling correction reaction during fictive locomotion in the cat. J Neurophysiol 94: 2053–2062, 2005. First published May 25, 2005; doi:10.1152/jn.00176.2005. In cat and humans, contact between an obstacle and the dorsum of the foot evokes the stumbling corrective reaction (reflex) that lifts the foot to avoid falling. This reflex can also be evoked by short trains of stimuli to the cutaneous superficial peroneal (SP) nerve in decerebrate cats during the flexion phase of fictive locomotion. Here we examine intracellular events in hindlimb motoneurons accompanying stumbling correction. SP stimulation delivered during the flexion phase excites knee flexor motoneurons at short latency [minimum excitatory postsynaptic potential (EPSP) latency 1.8 ms; mean 2.7 ms]. Although a similar short latency excitation occurs in ankle extensors (mean latency, 2.8 ms), recruitment is delayed until successive shocks in the stimulus train overcome the locomotor-related hyperpolarization of ankle extensors. In ankle flexor motoneurons, SP stimulation evokes an inhibition (mean latency, 2.7 ms) that briefly reduces or stops their firing during the flexion phase. There is a phase-dependent modulation of SP-evoked EPSP amplitude as well as latency during locomotion. However, the more obvious change in SP reflex pathways with the onset of fictive locomotion is the reduced inhibition of ankle extensor motoneurons and the increased inhibition of ankle flexors. These results show that the characteristic pattern of hindlimb motoneuron activation during SP nerve–evoked stumbling correction results from 1) di- and trisynaptic excitation of knee flexor and ankle extensor motoneurons; 2) increased inhibitory postsynaptic potentials in ankle flexors and a suppression of inhibition in extensors, 3) sculpting of the short-latency SP postsynaptic effects by motoneuron membrane potential, and 4) longer latency excitatory effects that are likely evoked by lumbar interneurons involved in the generation of fictive locomotion.

INTRODUCTION

In the cat, contact between the paw dorsum and an object during the swing phase of locomotion evokes a characteristic pattern of motoneuron activity that lifts the paw to clear the obstacle (Buford and Smith 1993; Forssberg 1979; Forssberg et al. 1977; Prochazka et al. 1978; Wand et al. 1980). Studies in chronic spinal cats (Forssberg 1979; Forssberg et al. 1977) indicate that the neuronal circuitry necessary to generate this functionally important reflex is contained within the lumbar spinal cord. During the stumbling corrective reaction, knee flexors are activated, and there is a brief period in which ankle extensors are excited. This is followed by an increased and often prolonged flexion at the hip, knee, and ankle (Buford and Smith 1993; Forssberg 1979; Forssberg et al. 1977; Pratt et al. 1996; Prochazka et al. 1978; Wand et al. 1980). The same stimulation during the extension phase evokes another reflex, the stumbling preventive reaction. During this reflex, ongoing extension is increased, and there is an increase in flexor motoneuron activity in the subsequent flexion phase (Buford and Smith 1993; Forssberg 1979).

The companion paper (Quevedo et al. 2005) describes the stumbling corrective and preventive reactions occurring during brain stem–evoked fictive locomotion in decerebrate cats. Short trains of shocks (10–25 pulses at 200 Hz, typically twice threshold) delivered to the SP nerve during the flexion phase of fictive locomotion evoke the same pattern of ipsilateral hindlimb motoneuron activity occurring during real stumbling correction. As recorded in the neurogram (ENG) this pattern includes 1) an increase in knee flexor motoneuron activity, 2) a brief burst of ankle extensor activity, and 3) an initial inhibition of ankle flexor activity, followed by 4) a prolonged excitation of hip, knee, and ankle flexor activity (Quevedo et al. 2005). The same stimulation delivered during the extension phase of fictive locomotion evokes the extensor excitation and subsequent increase in flexor motoneuron activity reported in intact preparations during stumbling preventive reactions (Bu ford and Smith 1993; Forssberg 1979).

The central pathways responsible for stumbling correction have not been studied. While the short latency effects from (usually) single shock SP stimulation in hindlimb motoneurons have been extensively described (Anderson et al. 1978; Burke 1999; Degtyarenko et al. 1996; Fleshman et al. 1988; Omeniuk 1990; Schmidt et al. 1988, 1989), it remains unclear how these actions relate to the generation of the stumbling corrective reflex. The goal of this study was therefore to use intracellular recording to characterize the synaptic actions occurring in hindlimb motoneuron pools during the stumbling corrective reaction. Specifically we wished to determine the synaptic events responsible for the initial suppression and excitation of ankle flexor activity during stumbling correction, the delayed recruitment of ankle extensors during the flexion phase, and the short latency excitation of knee (and sometimes hip) flexors. Some preliminary results have been presented (McCrea 2002; Stecina et al. 2003).

METHODS

Intracellular recordings from antidromically identified hindlimb motoneurons were obtained during fictive locomotion in 13/14 of the
RESULTS

Motoneurons were made using glass microelectrodes (1.6–2 MΩ) following guidelines set by the Canadian Council on Animal Care and the University of Manitoba. After surgical preparation using halothane anesthesia, a precollicular–postmamillary decerebration was performed. Anesthesia was discontinued, and the cat was paralyzed with gallamine triethiodide or pancuronium bromide and artificially ventilated.

Ipsilateral hindlimb nerves mounted for stimulation or recording included quadriceps (Q), sartorius (Sart), posterior biceps and semitendinosus (PBS), semimembranosus and anterior biceps (SmAB), medial gastrocnemius (MG), lateral gastrocnemius and soleus (LGS), plantaris (Plant), flexor digitorum longus (FDL), flexor hallucis longus (FHL), tibial, peroneus longus (PerL), tibialis anterior (TA), peroneus tertius and brevis, extensor digitorum longus (EDL), and superficial peroneal (SP). The SP nerve was stimulated with single shocks or with trains (5-ms interpulse interval; typically 15–25 shocks per train). SP stimulation also causes a strong activation of ankle flexors, including quadriceps (Q), sartorius (Sart), posterior biceps and semitendinosus (PBS), and the bifunctional (knee flexors and hip extensors, PBSt).

Intracellular recordings from 65 antidromically identified lumbar motoneurons were made using glass microelectrodes (1.6–2 MΩ) filled with 1.5 M potassium citrate. The sample consisted of 4 hip flexors (Sart), 3 hip extensors (SmAB); 8 knee flexor–hip extensors (PB or St), 2 knee extensors (Q), 1 rectus femoris, 14 ankle flexors, 28 ankle extensors, 3 FDL/FHL, 1 tibial, and 1 peroneus tertius or brevis motoneuron. To facilitate analysis of locomotor-related postsynaptic potentials, motoneuron action potentials were blocked in some experiments by adding 100 mM N-[2,6-dimethylphenylcarbamoyl-methyl]triethylammonium bromide (QX-314; Alamone Laboratories, Jerusalem, Israel) to the microelectrodes. In a few cases, intracellular recordings were made simultaneously from two motoneurons using independently positioned microelectrodes to facilitate direct comparisons of intracellular events in different motor pools during stumbling correction.

Figure 1A presents a typical example of a stumbling corrective reaction during fictive locomotion. Averaged, rectified–integrated ENG recordings obtained during a bout of mesencephalic locomotor region (MLR)-evoked fictive locomotion during control steps (dotted lines) are shown overlaid on records from steps in which a 15-shock train (200 Hz, 2 T) to the SP nerve evoked the stumbling corrective reaction (solid lines) during the flexion phase. Soon after the onset of SP nerve stimulation, there is an increase in activity recorded in hip flexor (Sart) and the bifunctional (knee flexors and hip extensors), PB and St, ENGs. Activity in the ankle flexors, PerL and TA, is initially suppressed and then increases after the end of the SP stimulus train. The increased activity of hip, knee, and ankle flexors persists well beyond the termination of SP nerve stimulation. SP stimulation also causes a strong activation of the ankle extensor, LGS, but not of the hip extensor, SmAB. In this example, the SP-evoked LGS activity during flexion is much larger than that occurring during the extension phase of the fictive step cycle. The onset of ankle extensor excitation is delayed from that of PB and St. Together these features show the fictive stumbling corrective reaction (Quevedo et al. 2005).

Modulation of SP-evoked postsynaptic potentials in a FDL motoneuron during the stumbling corrective reflex

The primary goal of this study was to describe the intracellular events occurring in the major motoneuron pools involved in removing the limb from an obstacle during stumbling correction. Therefore focus was placed on obtaining intracellular recordings from the principal ankle flexors and extensors and from the bifunctional knee flexors and hip extensors, PBSt. The FDL cell shown in Fig. 1 is shown because of the extensive literature on the effects of single shock SP stimulation in FDL motoneurons (see Introduction). Although it was the only antidromically identified FDL motoneuron recorded during stumbling correction, the intracellular events in this cell are similar to those occurring in other hindlimb motoneuron pools.

FIG. 1. Modulation of superficial peroneal (SP)-evoked postsynaptic potentials (PSPs) in a flexor digitorum longus (FDL) motoneuron during the stumbling corrective reaction. A: from top, averaged (n = 8) integrated and rectified neurogram (ENG) recordings from hip [sartorius (Sart) and semimembranosus and anterior biceps (SmAB)], knee [posterior biceps and semitendinosus (PBS)], ankle [tibialis anterior (TA) and lateral gastrocnemius and soleus (LGS)], and FDL nerves, and an intracellular (IC) recording from a FDL motoneuron. The SP nerve was stimulated during the flexion phase of mesencephalic locomotor region (MLR)-induced fictive locomotion with a 200-Hz train of 15 shocks at twice threshold intensity as indicated by the black bar and vertical dotted lines. Solid and dotted traces indicate averages obtained during stimulated and nonstimulated (control) cycles, respectively. B: intracellular recordings of the FDL motoneuron during flexion (bottom solid trace) and in the absence of locomotion (top dotted trace) during the period of SP stimulation (solid bar). C: expanded segments of the records in B (indicated by the bracket and dashed lines). Shortest central latencies of SP-evoked excitatory PSPs (EPSPs) were 2.0 and 1.8 ms in the absence of locomotion and during flexion phase, respectively. White arrowheads indicate the arrival at the cord dorsum of afferent volleys from the 1st 3 shocks of SP nerve stimulation.
The FDL ENG in Fig. 1A shows a very brief recruitment of motoneurons in the FDL motoneuron pool with the onset of SP stimulation. The rest of the response is similar to that seen in the PerL and TA ENGs with an inhibition of motoneuron activity during the stimulus train followed by a period of enhanced activity. The bottom trace in Fig. 1A is an intracellular record from an FDL motoneuron in which any action potentials that might have occurred were blocked by diffusion of QX314 from the recording electrode. The intracellular record shows an initial small and brief depolarization that quickly changes to a large hyperpolarization. After the end of the SP stimulus train, this hyperpolarization is replaced by a depolarization that is larger than that occurring without SP stimulation (dotted lines). These membrane potential changes are qualitatively similar to the sequence of initial recruitment, inhibition, and finally excitation of FDL motoneurons recorded in the rectified-integrated FDL ENG.

The intracellular records from this motoneuron are shown at expanded time scales in Fig. 1, B and C. The records in B show the control response to the 15-shock SP stimulus before locomotion (top trace, control) and during stumbling correction (flexion). Before locomotion, SP evokes a series of postsynaptic potentials that are predominately excitatory with a small hyperpolarizing component. The same stimulation during flexion results in pronounced hyperpolarization of the FDL motoneuron that overpowers any short latency depolarization. The top record in Fig. 1C shows the control response to the first three SP stimuli before the induction of fictive locomotion. The open arrows indicate the arrival of the SP nerve volleys at the cord dorsum. SP stimulation evokes an excitatory postsynaptic potential (EPSP; open arrow; latency, 2.0 ms) followed by an inhibitory postsynaptic potential (IPSP; filled arrow; 4.3 ms). During stumbling correction (flexion), a clear hyperpolarization follows the EPSP. During the flexion phase, EPSP and IPSP latencies decreased to 1.8 and 3.3 ms, respectively.

**SP-evoked inhibition in ankle flexors is enhanced during stumbling corrective reactions**

As shown in Fig. 2, in a TA (A and B) and an EDL (C and D) motoneuron, ankle flexor motoneurons receive a strong SP-evoked inhibition during stumbling correction similar to that recorded in the FDL cell in Fig. 1. The top traces in Fig. 2, A and C (from 2 experiments), show the control effects produced by a stimulus train to the SP nerve without fictive locomotion. In the TA motoneuron (Fig. 2A) an initial depolarization (latency, 2.6 ms) is followed by a small hyperpolarization after the first few shocks, whereas in the EDL cell (Fig. 2C), a modest hyperpolarization (latency, 1.9 ms) is followed by a series of what is likely a mixture of excitatory and inhibitory postsynaptic potentials that return the membrane potential to baseline after the first few shocks. During stumbling correction evoked in the flexion phase of fictive locomotion, the SP-evoked hyperpolarization in both motoneurons increases markedly. Even though the amplitude of the initial depolarization is enhanced during flexion in the TA motoneuron (cf. control and flexion records in Fig. 2A), subsequent hyperpolarization dominates the response. In the EDL motoneuron, the response remains hyperpolarized throughout the stimulus train.

The intracellular records obtained during stumbling correction in Fig. 2, A and C, are plotted at a slow time base as the top traces in Fig. 2, B and D, respectively. The accompanying ENG records show the characteristic excitation of ankle extensors (Fig. 2B, LGS; Fig. 2D, MG) and of the bifunctional St (Fig. 2D) during stumbling correction, as well as the increased flexor motoneuron activity (TA and Sart) after the end of the stimulus train. The intracellular responses to SP nerve stimulation during stumbling correction are a mixture of excitation and inhibition in TA (Fig. 2B) and a large hyperpolarization in the EDL motoneuron. As in Fig. 1, the shapes of the intracellular responses in each motoneuron are qualitatively similar to the changes in the firing of the homonymous motoneuron pools recorded in the ENG. Thus the TA ENG shows a brief excitation followed by reduced and then enhanced activity after the stimulus train. During the period in which the EDL mo-
toneuron (Fig. 2D, top trace) was hyperpolarized, activity in the EDL motoneuron pool fell silent.

During stumbling correction, the latency of the decrease in ankle flexor ENG activity [14 ± 2 (SD) ms; n = 8] during the stimulus train was longer than that of the IPSPs recorded in ankle flexor motoneurons (2.7 ± 1 ms; n = 12). This difference (examples in Figs. 1A, 2, B and D, and 5A) is likely explained by the need for the SP-evoked inhibition to summate (i.e., with successive shocks in the stimulus train) to overcome the locomotor-related depolarization of ankle flexor motoneurons during flexion (see DISCUSSION).

The hyperpolarization of ankle flexor and FDL motoneurons during stumbling correction was larger than the SP-evoked inhibition recorded in the absence of fictive locomotion (Figs. 1B and 2, A and C). Some increase in the magnitude of hyperpolarization is to be expected from the flexion phase depolarization of these motoneurons that would increase the driving potential for the IPSP. For example, the ~12-mV depolarization of the EDL motoneuron in Fig. 2C during the active (flexion) phase would increase IPSP amplitude appreciably during locomotion. The records from the TA motoneuron in Fig. 2A provide evidence, however, that there is also a premotorneuronal augmentation of SP-evoked IPSPs during the flexion phase. The ~2-mV difference in the control and locomotor membrane potential in Fig. 2A is unlikely to account for the substantial increase in hyperpolarization during locomotion. Both the increased inhibition and the increased initial excitation in this TA motoneuron (Fig. 2A) are in keeping with an increase in the excitability of SP activated interneurons during the flexion phase of fictive locomotion (see Burke 1999).

In TA motoneurons, the initial control response was a small excitation in four of five cases, with a latency of 1.9–4.4 ms that was often cut short by an inhibition (latency, 2.4–7.4 ms). The example in Fig. 2A was the largest SP-evoked excitation of an ankle flexor motoneuron encountered. Only one EDL motoneuron showed an initial EPSP; the first responses in the other seven were hyperpolarizing (Degtyarenko et al. 1998). During the flexion phase, IPSPs in TA and EDL dominated the response to SP stimulation, with the earliest IPSPs being recorded in EDL motoneurons (latencies, 1.6–2.6 ms). During flexion, two TA and one PerL motoneurons displayed an initial SP-evoked EPSP. Table 1 presents a summary of EPSP and IPSP latencies recorded in the absence of locomotion and during the flexion phase. Pooling results from TA and EDL motoneurons, IPSPs during locomotion decreased in latency in some cells but increased in others. Thus the mean IPSP latency was not significantly different (P > 0.05) in ankle flexors between control (2.2 ms) and the flexion phase (2.7 ms).

### Short-latency SP-evoked EPSPs in knee and hip flexor motoneurons during fictive stumbling correction

During fictive and real stumbling correction, the bifunctional PB and St motoneurons are activated at short latency (Figs. 1A, 2D, and 4, A and C) (Quevedo et al. 2005). Unlike the brief initial excitation of ankle flexor and FDL motoneurons, PBSt excitation can persist throughout the SP stimulus train. Figure 3A shows an example of averaged ENG records obtained during stumbling correction with an intracellular record from a PBSt motoneuron. Soon after the onset of SP stimulation, PBSt activity increases (latency of 4 ms) and remains above levels seen in control steps without stimulation (dotted lines) after the stimulus train ends. The expanded records in Fig. 3B show the rapid onset of depolarization recorded in this motoneuron (latency of 2.7 ms from the arrival of the volley at the cord dorsum indicated by the vertical dashed line). A similar rapid depolarization is illustrated in another PBSt motoneuron in Fig. 4B (top trace). SP-evoked postsynaptic effects in PBSt remained depolarizing throughout the duration of the stimulation in seven of the eight motoneurons examined.

In the absence of locomotion, the average latency of SP-evoked excitation in PBSt motoneurons was 2.3 ms (Table 1). During the flexion phase, the mean EPSP latency was 2.7 ± 0.6 ms (n = 8) with two values <2.1 ms. The mean latency of PBSt motoneuron activation recorded as an increase in ENG activity was 4 ± 1 ms (Quevedo et al. 2005). Because the interpulse interval in the stimulus train was 5 ms (200 Hz), even the first shock in the SP stimulus train was often sufficient to recruit PBSt motoneurons during stumbling correction.

As seen in the ENG, the hip flexors Sart and Psaos are sometimes recruited during stumbling correction at latencies similar (mean, 4 ms) to those seen in PBSt. In some cases, hip flexor recruitment follows an initial inhibition (Pratt et al. 1996; discussed in Quevedo et al. 2005). Intracellular recordings from four Sart motoneurons in four preparations found a similar variability in short-latency SP-evoked excitation, with two cells showing a mixture of inhibition (latencies of 2.4 ms or longer) and excitation (latencies of 4.3 and 3.4 ms) and two showing only inhibition (latency, 2.8 and 3.1 ms). No intracellular recordings were made from Psaos motoneurons.

### SP-evoked excitation of extensor motoneurons

The latency of activation of ankle extensor motoneurons recorded in the ENG (16 ms) is consistently longer than that of PBSt motoneurons (4 ms; Quevedo et al. 2005; compare LGS and St ENG records in Fig. 1A). Initially this suggested to us the possibility of a longer latency excitatory reflex pathway from SP afferents to knee extensors than to knee flexor motoneurons. Intracellular recordings (12 MG, 9 LGS, 3 GS, and 4 Plant) during stumbling correction, however, revealed

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**TABLE 1. Latencies of EPSPs and IPSPs evoked by 2-T SP nerve stimulation**

<table>
<thead>
<tr>
<th></th>
<th>EPSPs</th>
<th>IPSPs</th>
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<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
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<tr>
<td>Control</td>
<td></td>
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<tr>
<td>MG, LGS, PL</td>
<td>2.2 ± 0.8 (16)</td>
<td>1.4–4.8</td>
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<tr>
<td>TA, EDL, PerL</td>
<td>2.7 ± 1.0 (6)</td>
<td>1.9–4.4</td>
</tr>
<tr>
<td>PB, St</td>
<td>2.3 ± 0.3 (3)</td>
<td>2.1–2.7</td>
</tr>
<tr>
<td>Flexion</td>
<td></td>
<td></td>
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<tr>
<td>MG, LGS, Plant</td>
<td>2.8 ± 1.7 (23)</td>
<td>1.4–13.8</td>
</tr>
<tr>
<td>PB, St</td>
<td>2.7 ± 0.6 (8)</td>
<td>1.8–3.7</td>
</tr>
<tr>
<td>TA, EDL, PerL</td>
<td>3.1 ± 1.0 (3)</td>
<td>2.4–4.4</td>
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Values are means ± SD with number of responses in parentheses. EPSP: excitatory postsynaptic potential; IPSP: inhibitory postsynaptic potential; MG: medial gastrocnemius; LGS: lateral gastrocnemius and soleus; Plant: plantar’s (Plant); TA, tibialis anterior; EDL, extensor digitorum longus; PerL, peroneus longus; PB, posterior biceps; St, semitendinosus; Plant, plantaris.
motoneuron, the first action potential occurred after the fifth from subsequent shocks in the stimulus train summate to recruitment. As shown in the intracellular traces, the EPSPs activity recorded in the ENG (i.e., a delay in LGS motoneuron ization is 2.0 ms (arrow, bottom trace), there is a delay in the bottom motoneuron (mot)) from six stumbling correction trials. Note that, although the intracellular latency of SP-evoked depolarization is 2.0 ms (arrow, bottom trace), there is a delay in the bottom motoneuron (mot). As shown in the intracellular traces, the EPSPs from subsequent shocks in the stimulus train summate to produce an increasing depolarization of the motoneuron. In this motoneuron, the first action potential occurred after the fifth SP-evoked EPSP. As judged from the ENG records, other LGS motoneurons were recruited with the third or fourth EPSP in the train. When stumbling correction is evoked during the flexion phase of the step cycle, extensor motoneurons are hyperpolarized. In comparison with the already depolarized PBSt motoneurons, EPSP summation is needed to bring ankle extensor motoneurons to threshold during stumbling correction. Thus differences in motoneuron membrane potential, and not differences in interneuronal path lengths, produce the differences in the latencies of PBSt and ankle extensor motoneuron activation during stumbling correction.

This conclusion is further supported by the examples shown in Fig. 5. When delivered during the flexion phase, SP stimulation evokes the stumbling correction reaction described above with short-latency excitation of PBSt and delayed excitation of ankle extensors, GS. Delivering the same stimulation during the extension phase produces the stumbling preventive reaction during real (Buford and Smith 1993; Forssberg 1979) and fictive locomotion (Quevedo et al. 2005). These two
Because of the variable and weak recruitment of hip (SmAB) and knee extensors (Q) motoneurons during fictive stumbling correction (Quevedo et al. 2005), few of these motoneuron species were studied with intracellular recordings. Three of the four Q or SmAB motoneurons tested at rest displayed an initial SP-evoked inhibition. During flexion, SP-evoked EPSPs (range, 1.7–16.7 ms) occurred in all five (2 Q and 3 SmAB) motoneurons examined.

**Modulation of SP-evoked synaptic responses in ankle extensor motoneurons during locomotion**

Unlike the consistently depolarizing actions of SP stimulation on PBSt motoneurons under control and locomotor conditions, SP stimulation was largely inhibitory to ankle extensors in the absence of locomotion. This is shown in Fig. 6A, where the averaged effects of SP stimulus trains are shown during control (dotted) and during the flexion phase (solid) of fictive locomotion for 15 ankle extensor motoneurons. While some of the traces in the top overlay in Fig. 6A show depolarization, inhibitory and mixed effects predominate at rest. During the flexion phase of fictive locomotion, SP stimulation evokes an overall depolarization in all but one motoneuron. The records in Fig. 6B were extracted from those in Fig. 6A to show a variety of SP-evoked postsynaptic potentials during control and flexion in ankle extensor motoneurons. In all four cases, a reduced inhibition and an increased excitation occurred with the transition to locomotion.

The locomotor-dependent facilitation or emergence of SP-evoked excitation in ankle extensors is shown again in Fig. 7. It shows overlaid averaged effects of single SP shocks under control (dotted) conditions and during the flexion (solid) and extension (dashed) phases of fictive locomotion. In all three ankle extensor motoneurons (Fig. 7, A–C), the dominant effects were inhibitory in the absence of fictive locomotion (IPSP latencies of 3.0, 3.4, and 2.7 for A, B, and C, respectively). The latency of the small initial EPSP in the MG motoneuron was only partially suppressed during locomotion, and a
longer latency excitation (12 ms) appeared during flexion (solid trace). Unlike the effects of single shock SP nerve stimulation shown, trains of stimuli to the SP nerve resulted in action potentials in this motoneuron during stumbling correction (data not shown). In six of eight ankle extensor motoneurons (3 LGS and 3 MG) tested with single shock stimulation, SP-evoked EPSPs were similar or larger during the flexion and extension phases of fictive locomotion. The MG motoneuron in A showed inhibition at rest (latency, 3.0 ms) that reversed to excitation during the flexion and extension phases of fictive locomotion. B: this MG motoneuron showed a mixture of EPSPs and IPSPs in the absence of locomotion (latencies of 2.5 and 3.4 ms, respectively) and only depolarization during flexion and extension (latency of 2.5 ms). C1 and C2: simultaneously obtained recordings from an LGS and an EDL motoneuron from another experiment. Same format as in A and B. Latencies of the IPSPs recorded in the LGS and EDL motoneurons without locomotion and during flexion and extension were 2.7 and 2.2 ms, respectively. During flexion, there was a reversal to an excitation occurring at 12.2 ms in the LGS motoneuron (solid trace in C1), and the inhibition of the EDL motoneuron recorded at rest increased (solid trace in C2). Membrane potential of the EDL motoneuron was −45 mV at rest and during extension and −38 mV during flexion.

**Discussion**

As shown in the companion paper (Quevedo et al. 2005), properly timed trains of stimuli to the cutaneous SP nerve can evoke the stumbling correction and preventive reactions in decerebrate cats during fictive locomotion. This study is the first to describe the intracellular events in several hindlimb motoneuron pools that accompany these reflexes. Because the main features of motoneuron activation during these functionally important reflexes seem similar in fictive and real locomotion (Quevedo et al. 2005), these results also provide a framework for understanding the synaptic events in motoneurons that result in lifting the foot over an obstacle to avoid tripping in intact preparations. Similarities between the stumbling reaction described here and in humans (Lam et al. 2003; Schillings et al. 1996; Zehr et al. 1997) include the increase in ongoing flexor activity, and when using an electrical stimulus train in adults, inhibitory effects in TA as well as a facilitation of ankle extensor EMG (Zehr et al. 1997).

Motoneuron activation and inhibition during the fictive stumbling corrective reaction can be divided into two stages. The first occurs during the SP stimulus train. In ankle flexors, there can be a brief excitation soon after stimulus onset, but the main effect is an inhibition that reduces or stops the flexion phase–related firing. At the same time, there is an oligosynaptic depolarization and recruitment of the bifunctional (mainly knee flexor) PBSt motoneurons and of ankle extensors. These results show that these early effects result from the summation of short-latency reflex pathways from SP afferents to ipsilateral hindlimb motoneurons. The second stage of stumbling correction, which may begin earlier but often follows the SP stimulus train, is an enhancement of flexor motoneuron activity in pools operating at the hip, knee, and ankle. Depending on stimulus conditions, this enhanced flexor activity may extend the duration of the ongoing flexor phase (Quevedo et al. 2005). For the stumbling preventive reaction evoked during extension, the second stage includes an increase in ongoing extensor activity throughout the limb and often an increase in flexor motoneuron activation during the subsequent flexor phase.

**Central latencies of the SP-evoked synaptic responses**

The minimum latencies of SP-evoked EPSPs were 1.4–1.6 ms in ankle extensor and PBSt motoneurons (mean values in Table 1). According to the detailed studies by the Burke laboratory, latencies of cutaneous EPSPs <2.1 ms (and under some conditions, <2.3 ms) are likely to be mediated through a disynaptic pathway (Burke et al. 2001; Degtyarenko et al. 1998). Forssberg et al. (1977), using indirect measurements, estimated the central latency of St excitation to be 2.2 ms during real stumbling correction, a value similar to that reported here. Thus the reflex pathways responsible for the first effects in motoneurons during stumbling correction include short-latency excitation and inhibition in hindlimb motoneurons mediated by di- and/or trisynaptic reflex pathways (i.e., consisting of 1 or 2 interneurons) interposed between cutaneous afferent fibers and motoneurons. In general terms, the net effect of SP stimulus trains during stumbling correction was an inhibition of ankle flexors and an excitation of ankle extensor and PBSt motoneurons. These net effects were sometimes unlike the initial de- or hyperpolarizing components (e.g., Figs. 1, 2, and 6) and point out the need to examine the effects of trains of stimuli to determine the functional consequences of cutaneous nerve stimulation in individual motoneurons (e.g., Heckman et al. 1992; Perrier et al. 2000).
The well-documented decrease in SP-evoked EPSP latency during locomotion in FDL motoneurons has been attributed to increased interneuronal excitability (Burke 1999; Burke et al. 2001; Moschovakis et al. 1991; Schmidt et al. 1988). Reduced latencies of some SP-evoked EPSPs were also seen in this study (e.g., Fig. 1C). The average latencies of the SP-evoked EPSPs were not, however, shorter during locomotion in ankle extensors or in knee flexors. Thus central modulation of reflex pathway latency is not a prominent component of the reflex excitation underlying stumbling corrective and preventive reactions. Although no systematic comparison was made, SP-evoked EPSP latencies in extensor motoneurons were similar in the flexion and extension phases (Figs. 5, B and D, and 7, A and B).

On the other hand, the amplitudes of short latency SP EPSPs in the TA motoneuron in Fig. 2A and in some of the ankle extensors motoneurons in Fig. 6 were often facilitated during locomotion. Increases in the size of SP-evoked EPSPs during fictive locomotion have been reported before (Anderson et al. 1978; Schmidt et al. 1988, 1989) and attributed to increased excitability of SP-activated interneurons (see Burke 1999). Presumably this increased excitability more than counters the presynaptic reduction in synaptic transmission from cutaneous afferents to their spinal targets that occurs during fictive locomotion (Gossard et al.1989; Perreault et al. 1999). These results show that the initial activation of TA (and FDL) motoneurons is produced by the depolarizing component of SP-evoked postsynaptic potentials. Because the net effect of SP stimulation is inhibitory, this excitation is short-lived and replaced by a prominent inhibition that can silence ankle flexor ENG activity during the stimulus train (Fig. 1).

Shaping and modulating SP reflexes during fictive locomotion

Given the delay between the onset of increased PBSt ENG activity (latency of 4 ms) and the onset of ankle extensor ENG activity (latency of 16 ms), we were initially surprised to find that the intracellular latencies of SP-evoked excitation were similar in ankle extensor and PBSt motoneurons. The explanation for this delay seems to be simply the difference in membrane potential in these motoneuron pools during the flexion phase of the locomotor cycle. In PBSt motoneurons, the locomotor-related depolarization during flexion allows SP-evoked excitation to bring the membrane potential to threshold quickly. PBSt motoneuron firing often occurs with the first shock in the stimulus train. In ankle extensors, EPSP summation with several shocks is required to overcome the locomotor-related hyperpolarization during the flexion phase and to bring these motoneurons to threshold. The same stimulation applied during the extension phase when extensor motoneurons are depolarized (i.e., during stumbling prediction) results in a short-latency increase of ongoing activity in extensor motoneuron ENGs (Fig. 5C; 3 ms) (Quevedo et al. 2005). During flexion phase-evoked stumbling correction, there is a delay between the onset of SP nerve stimulation and the reduction in ankle flexor motoneuron firing. This can also be explained by the need for IPSP summation to bring the membrane potential below firing threshold in these motoneuron pools. Thus motoneuron membrane potential plays an important role in sculpting the patterns and latencies of activity during stumbling correction.

Another consideration in the reflex recruitment of motoneurons during stumbling correction is the change in motoneuron excitability that occurs during fictive locomotion. In the decerebrate preparations employed here, there is a substantial (mean, 8 mV) hyperpolarizing shift in the voltage threshold for action potential initiation in both flexors and extensors (Krawitz et al. 2001). This shift would substantially increase the possibility of small-amplitude, SP-evoked EPSPs bringing motoneurons to threshold.

These observations also offer an explanation for the failure of single electrical shocks to evoke stumbling correction in intact preparations (summarized in Buford and Smith 1993). Single-shock, SP nerve stimulation does not stop ongoing activity in ankle flexors (e.g., Degtyarenko et al. 1998) nor does it result in the recruitment of ankle extensors (Fig. 7) during fictive stumbling correction. This contrasts the powerful inhibition of ankle flexors and excitation of ankle extensors seen when the SP nerve was stimulated with trains. These results suggest that trains of stimuli are needed to overcome locomotor-related motoneuron depolarization and hyperpolarization to produce inhibition of ankle flexor (Fig. 2) and excitation of ankle extensor motoneurons (Figs. 4 and 5), respectively. Forssberg (1979) showed that, when stimulation is increased from one to several pulses during stumbling correction, there is a large increase in St activity.

The initial excitation of TA motoneurons during real stumbling correction in cats had been attributed to monosynaptic excitation by muscle spindle afferents during obstacle contact (Prochazka et al. 1978). These results show that this initial motoneuron recruitment can be evoked by activation of cutaneous afferents alone. Our view is that the stumbling corrective reactions evoked during the swing and stance phases are cutaneous reflexes. Because they can be fully activated by only a limited set of afferents, we think that they are examples of a “private” reflex pathway originally described for reflexes around the foot (Engberg 1964; Hongo et al. 1990) or of “local sign” (Hagbarth 1952) and not flexion reflexes (see McCrea 1992). The details of the responses evoked during stumbling correction will, however, depend on the complement of afferents activated. The companion paper discusses additional reflex effects that might be evoked by muscle afferents also activated during stumbling correction (Quevedo et al. 2005).

Reflex pathways mediating SP reflexes during the stumbling corrective response

Figure 8 presents a hypothetical organization of spinal interneuronal pathways involved in stumbling correction. The flexor and extensor portions of the central locomotor pattern generating circuitry (CPG) are represented by the E and F in the circle at the top of the figure. Filled circles denote inhibitory interneurons, and open circles denote excitatory interneurons. The number of interneurons in these cutaneous pathways is based on the estimates of latencies obtained in this study, the work of Burke et al., and the assumption that each synapse contributes a delay of about 0.8-1.0 ms (Burke 1999; Burke et al. 2001).

SP afferents contact the CPG and two sets of excitatory interneurons (•): one projecting directly to motoneurons (E-2)
The phasic modulation of short-latency SP excitatory pathways in inhibitory interneurons is not shown in Fig. 8. Simplicity, a disynaptic inhibition with direct activation of suggest mediation by di- and trisynaptic pathways. For this reason (Fig. 2) at latencies as short as 1.6 ms (2.1 ms excitation and are strongly inhibited during stumbling correction. These pathways suppress ankle flexor activity for the currently active portion of the CPG circuitry (thick line). Phasic modulation of interneurons mediating di- and trisynaptic excitation from SP afferents is indicated by the dotted line from the CPG. For simplicity, the weak excitation of hip and knee extensors is not shown. The inhibitory pathway to ankle extensor motoneurons observed in the absence of locomotion (last-order inhibitory interneuron 2) is inhibited by the locomotor circuitry.

and the other exciting the last-order excitatory interneurons (E-1). The ankle flexors, TA and PerL, hip flexors, and FDL motoneurons receive di- and trisynaptic excitation through these excitatory interneurons. The same motoneurons receive a three and four synapse-mediated inhibition. For simplicity the inhibitory interneurons projecting to flexors and FDL (I-1) are activated by the same interneurons that excite ankle extensors and flexors. Trains of stimuli to SP result in a disynaptic excitation and longer latency inhibition corresponding to the effects recorded intracellularly in this study. During stumbling correction, these pathways suppress ankle flexor activity for the duration of the stimulus train. The results for the FDL motoneuron in Fig. 1 show that inhibition can dominate even in the presence of a large initial excitatory component in the postsynaptic potential. EDL motoneurons lack the SP-evoked excitation and are strongly inhibited during stumbling correction (Fig. 2) at latencies as short as 1.6 ms (2.1 ± 0.3 ms), suggesting mediation by di- and trisynaptic pathways. For simplicity, a disynaptic inhibition with direct activation of inhibitory interneurons by SP afferents is not shown in Fig. 8. The phasic modulation of short-latency SP excitatory pathways (Burke 1999) is indicated by the dotted line from the CPG to excitatory interneurons in Fig. 8.

An important finding of this study is that the SP-evoked inhibition of ankle extensor motoneurons is suppressed during locomotion (Figs. 6 and 7). This is shown in Fig. 8 by the CPG-evoked inhibition of the population of inhibitory interneurons contacting ankle extensor motoneurons (I-2). As a result of this disinhibition, the effects of SP stimulation during locomotion are strongly depolarizing. On the other hand, the inhibition in ankle flexor motoneuron is increased during stumbling correction. While this may partially result from an enhancement of the driving potential for IPSPs by motoneuron depolarization during the flexion phase, substantial increases in IPSP amplitude can occur with minimum motoneuron depolarization (Fig. 2). In accordance with the conclusions of others (Burke 1999; Degtyarenko et al. 1996), we suggest that there is a premotoneuronal facilitation of transmission in the inhibitory pathway from SP afferents to ankle flexor motoneurons during the flexion phase of fictive locomotion. This could result from either a direct, CPG-mediated excitation of these inhibitory interneurons, or as shown in the figure, from the increased activity in the excitatory SP-activated interneurons. While direct experimental data are lacking, these results strongly argue for at least two populations of inhibitory interneurons in these SP reflex pathways (I-1 and I-2; Fig. 8).

Stumbling correction evoked during the flexion phase results in a strong activation of hip, knee, and ankle flexor motoneurons. Because these effects are often largest after the end of the stimulus train, it is unlikely that this widespread flexor excitation is evoked through the di- and trisynaptic SP reflex pathways discussed thus far. Instead, it is more likely that SP afferents evoke some of their actions during stumbling correction by accessing the pattern generating circuitry producing locomotion. CPG-mediated effects would explain the changes in cycle phase duration or timing that can accompany stumbling correction (Figs. 2B and 5, A and C) (Quevedo et al. 2005). An excitation of CPG circuitry would also explain the ability of SP stimulation during the extension phase to increase flexor activity in the subsequent flexor phase (Fig. 5C) during the stumbling preventive reaction (Quevedo et al. 2005). While long-latency responses could theoretically be generated through long-loop reflexes to supralumbar centers, long-latency stumbling reflexes in St motoneurons are also observed in low spinal cats (Forssberg 1979; Forssberg et al. 1977). In Fig. 8, SP afferents are shown to contact both extensor and flexor portions of the CPG. The implication is that effects evoked through the CPG would depend on the current locomotor phase. Thus activation of SP afferents during the flexion phase would excite flexor portions of the CPG resulting in increased hip, knee, and ankle flexion to remove the foot from the obstacle. The same stimulus during extension would increase CPG-generated stance activity and increase forward propulsion.

Figure 8 presents a first attempt to describe the pathways responsible for the functionally important stumbling correction reaction. It also suggests a number of predictions about the nature of the neurons responsible for stumbling correction and prevention. For example, using extracellular interneuron recordings, it should be possible to distinguish the two populations of inhibitory interneurons in Fig. 8 from their axonal projections using antidromic activation from within the ankle.
extensor or flexor motor nuclei (Angel et al. 2005) and the locomotor-related inhibition and facilitation of those projecting to ankle extensors and flexors, respectively. Antidromic stimulation from the motor nuclei could also help determine if the same interneurons project to PBSt and ankle extensor motoneuron pools. The phasic modulation of SP-evoked EPSPs during locomotion suggests that either the first- or last-order excitatory interneurons could be rhythmically active during the flexion phase. Finding such activity would offer experimental support to the idea that some of the last-order interneurons mediating SP reflex responses are also used by the spinal generator for distributing excitation to motoneurons during locomotion (Anderson et al. 1978; Burke 1999; Burke et al. 2001). A dual role in reflex and CPG-related excitation of extensor motoneurons has been recently postulated for last-order interneurons in disynaptic reflex pathways from extensor group I muscle afferents (Angel et al. 2005). While direct recordings from interneurons will be needed to test these hypotheses, these results offer insight into how segmental reflex pathways, changes in motoneuron excitability, and the locomotor CPG cooperate to produce functional reflexes.

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