Sustained Firing of Alpha and Gamma Hind Limb Motoneurons Induced by Stimulation of the Pudendal Nerve

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Cueva-Rolón, Rafael, Rodolfo Delgado-Lezama, J. G. Raya, M. Raya, R. Tecuanhuey, and E. J. Muñoz-Martínez. Sustained firing of alpha and gamma hind limb motoneurons induced by stimulation of the pudendal nerve. J Neurophysiol 88: 3232–3242, 2002; 10.1152/jn.00157.2002. Axons from receptors in the cat vaginal wall run in the sensory pudendal nerve (SPN), and brief (<10 s) vaginal probing (VP) in the decerebrate cat produces a long-lasting (>1 min) contraction of the triceps surae (TS) muscles. The aim of the present project was to find out whether brief SPN stimulation also produces sustained TS response and, eventually, to study the mechanisms involved in it. Decerebrate female cats were used. In some cats, TS electromyography (EMG) and tension response were recorded; stimulation of left SPN with single or repetitive trains of shocks produced a bilateral TS response that outlasted the stimulus >1 min as VP did. In paralyzed cats (pancuronium; Panc), intracellular recordings were made from hind limb motoneurons (MNs). SPN stimulation produced a depolarization ≤5 s long and occasional cell firing only lasting <2.5 s; this is in contrast with the prolonged TS postdischarge seen in nonparalyzed cats. If MNs were depolarized below the firing threshold by current injection, about half of them showed bistable firing that could last several minutes in response to SPN train. It is suggested that MNs might hyperpolarize after Panc injection. Before Panc injection, SPN train produced long-lasting (>1 min) electromyographic (ENG) postdischarge in a small filament of the medial gastrocnemius (MG) nerve; the MG EMG postdischarge was also recorded. Large spikes (LS) and small spikes (SS) were distinguished in the ENG. During the postdischarge, LS frequency and the integrated EMG activity correlated well ($r > 0.9$); no correlation was found between SS and EMG. After Panc injection, LS postdischarge was absent but the SS postdischarge remained. LS followed by EMG potential were also evoked by brief TS stretch (reflex LS); single shocks to SPN only elicited SS that were not followed by EMG potential. It is concluded that alpha axons and gamma axons produced LS and SS, respectively, and that SPN activates gamma axons. It is proposed that, in the nonparalyzed cats, the stimulation of SPN with trains of shocks might cause an increase in the afferent inflow from muscle spindles to alpha MNs through the sustained firing of gamma MNs. The increased excitatory inflow would depolarize alpha MNs and allow bistable MN firing; Panc would decrease this inflow by blocking transmission to the spindle fibers.

INTRODUCTION

Vaginal probing (VP) in the cat activates axons in the sensory pudendal nerve (SPN) (Cueva-Rolón et al. 1994). In the decerebrate cat, VP induces sustained firing of previously silent motor units of triceps surae muscles (Cueva-Rolón et al. 1993); brief vibration of the Achilles tendon induces similar firing (Crone et al. 1988). In both cases, the firing may outlast the stimulus by a few minutes. On the other side, in previously silent motoneurons (MNs), a brief intracellular current pulse causes persistent firing after the pulse; this is known as MN bistable behavior (MN BB in this paper) (Hounsgaard and Kiehn 1985; Hounsgaard et al. 1984, 1988; see also Bennett et al. 1998a,b; Hultborn 1999; Kiehn 1991; Lee and Heckman 1998a,b; Paroschy and Shefchyk 2000). MN BB results from the activation of a persistent inward current (Schwindt and Crill 1977) and causes the sustained firing in response to tendon vibration and might also cause the response to VP.

MN BB depends on the MN membrane potential (MP). If the MP is well below the firing threshold, a voltage pulse that reaches this threshold only causes cell firing during the pulse (Hounsgaard et al. 1984, 1988). The MN MP depends in part on the excitatory inflow from muscle spindle afferents; this inflow is regulated by gamma MNs. Thus MN BB might be conditioned by the activity of gamma MNs. In response to a brief input, these MNs might show persistent firing as alpha MNs do.

This work had three aims. The first was to find out if SPN stimulation induces motor unit firing similar to that evoked by VP, second to test whether SPN stimulation triggers BB in hind limb MNs, and third to test whether gamma MNs show postdischarges in response to SPN stimulation.

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METHODS

Experiments were performed in 39 female cats weighing 2.5–3.4 kg. Surgical procedures were performed under ether anesthesia. The respiratory movements and the electrocardiograph (EKG) were continuously monitored. The lack of pupil reaction to surgical maneuvers was checked frequently. Once the minor surgery was completed (cannulation of the trachea and the radial vein, and nailing the femur and the fibula), the cats were decerebrated by brain stem transection.

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at the intercollicular level; the forebrain was removed. Then the anesthesia was discontinued, and the surgery was completed. The cats included in the present report showed decerebrate rigidity. In all experiments, the sensory pudendal nerve (SPN) was exposed and sectioned at the ischiatic fossa; the central stump of SPN was mounted on hook (Ag-AgCl) electrodes for stimulation.

**Recording muscle tension, EMG, and ENG**

In 14 experiments the cats were not paralyzed. The insertion of the Achilles tendon on the calcaneum was kept intact. In these cats, the left leg was tightly fixed and tension was recorded with a strain-gauge that was applied against the process of the calcaneum as previously described (Cueva-Rolón et al. 1993). In eight cats, electromyographic (EMG) recordings were taken from one or more muscles of the TS complex. In seven other cats, electroneurography (ENG) was also taken from the central stump of a sectioned MG nerve filament; unitary ENG spikes could be distinguished in three experiments. Reflex ENG and EMG potentials were elicited by controlled taps that were applied to the calcaneum (see Cueva-Rolón et al. 1993). The electrical and the mechanical responses of TS muscles to the SPN stimulation were recorded; the duration of the electric shocks was 50 $\mu$s; other details on the stimuli are given in the appropriate section of RESULTS. In two cats, the right SPN and the left sural nerve were also stimulated. In three other cats, the effect produced by the posterior femoral cutaneous nerve (PFCN) and the genital nerve (GenN) was tested. The effects of SPN stimulation were studied in three cats before and after the spinal cord was transected ($T_{12}$).

**Detection of ENG spikes. Statistical analysis**

ENG was captured on-line and stored in a computer using two software programs (Axotape v 2.0.2; Axoscope 8). Unitary spikes of very different amplitudes were seen in the ENG. When the TS muscles were slack and the EMG of the MG muscle was flat, only small spikes (SS) were detected; when the muscle was stretched, EMG activity appeared and the SS frequency increased, but then larger spikes (LS) also appeared. The amplitude distribution as well as the firing frequency of the spikes were analyzed using two other programs (Microcal Origin Version 6.0; Microsoft Excel 97). The minimal amplitude of SS (SSmin) that was considered for frequency counting was fixed as twice the amplitude of the background noise (Fig. 1); therefore if smaller spikes occurred, they were excluded. Then the amplitude distribution of SS was determined when only these spikes were present in the ENG (the slack muscle with flat EMG; see RESULTS). The minimal amplitude of LS (LSmin) was larger than the maximal amplitude of SS (SSmax); the latter was determined as

$$SS_{\text{max}} = SS_{\text{avg}} + 2\sigma$$

$\sigma$ being the standard deviation (SD) of SS distribution. LSmin was taken as

$$LS_{\text{min}} = SS_{\text{avg}} + 3\sigma$$

The amplitude of near 2% of the spikes was between SSmax and LSmin; these spikes were not considered.

The spike frequency was determined in successive 5-s-long samples. The frequency of all spikes ($F_{\text{all}}$; amplitude larger than SSmin) was determined first. Afterward, the LS frequency ($F_{\text{LS}}$) was determined (spike amplitude $\geq$ LSmin). Finally, SS frequency, $F_{SS} = F_{\text{all}} - F_{\text{LS}}$.

**Intracellular recordings**

The lumbar and the sacral segments of the spinal cord were exposed in 18 cats. The $L_4$ dorsal root as well as the $L_7$ and $S_1$ ventral roots were dissected and cut as close as possible to the their exit from the vertebral canal. SPN, posterior biceps and semitendinous (pBSt); triceps surae (TS); deep peroneal (DPer) and tibial (Tib) nerves were dissected and cut on the left side. The dissected ventral roots and nerves were placed on bipolar hook electrodes for stimulation. Pools that were made with the skin flaps were filled with mineral oil at 36–37°C. The pools temperature was maintained by radiant heat. The cats were paralyzed by an intravenous injection of pancuronium (80 $\mu$g/kg, plus 40 $\mu$g when needed); artificial respiration was installed and bilateral pneumothorax was performed. Using conventional techniques, intracellular recordings of spinal neurons were made in the $L_7$ segment in paralyzed cats. Glass micropipettes (12–20 $M\Omega$) that were filled with potassium acetate (2.7 M) were used. Spinal motoneurons were identified by their response to ventral root stimulation and by the monosynaptic potential that was elicited by stimulation of a muscular nerve. Recordings of MNs were displayed in an oscilloscope and photographed or captured on-line in a computer for further analysis.

Additional details are given in RESULTS. At the end of the experiments an overdose of pancuronium was given and the artificial respiration was suspended to kill the cats. Hind limb nerves were dissected in continuity with the spinal roots $L_7$ and $S_3$ to measure the conduction distance.
RESULTS

Motor response to stimulation of SPN in nonparalyzed cats

VP produces a sustained response of TS muscles (Cueva-Rolón et al. 1993). It was proposed that this response might be triggered by impulses in the SPN (Cueva-Rolón et al. 1994); this was tested in the present experiments. The left SPN was sectioned and the central nerve stump was stimulated. The SPN afferent volley was recorded in the cord dorsum at S2 dorsal root level; the threshold \( T \) using 50 \( \mu \)s shocks was 2.9–3.1 V. SPN was stimulated with trains of shocks lasting \( \approx \)200; the shocks and the train frequency were 100–150 and 1–5 Hz, respectively. In most trials, one to six trains of 10–20 shocks were used, but lower shock frequencies or single shocks were applied when small responses were needed. In 14 cats, TS EMG and tension response was produced by SPN stimulation. In five of these cats, a single train produced a response that started before the end of the train and lasted \( >1 \) min (Fig. 2A). In nine cats, three to six trains were needed to produce a maximal response; the response to each train was delayed by 50–500 ms with respect to the end of the train, and gradually increased in amplitude (Fig. 2B). The long delay of the TS response can be explained by the slow rising depolarization and delayed cell firing that can be induced in TS MNs by train stimulation of SPN (see following text and Fig. 5B). The gradual increase of the TS response might result from intrinsic facilitation (wind up) in interneurons (Ints) (Mendell 1966; Russo and Hounsgaard 1994).

We wondered whether skin nerves that do not innervate the pubic or the pudendal areas might also produce sustained contraction of the TS muscles. To answer the question, the distal third of the sural nerve was stimulated \( n = 2 \) with shocks of intensity up to five times larger than the intensity of the shocks used to stimulate SPN. This stimulation only produced small and transient contractions of TS muscles (Fig. 2C).

The long-lasting contraction of previously quiescent TS muscles could reflect the bistable behavior of MNs (MN BB), which is not expressed in the spinal cat (Hounsgaard et al. 1988). Thus if the sustained TS contraction shown here resulted from MN BB, it may be expected that sectioning the spinal cord would prevent or terminate the contraction. In two cats, the spinal cord was sectioned during a sustained TS contraction, which ceased immediately after the section; later a stronger stimulation of SPN was ineffective (Fig. 2D).

VP produces a bilateral motor response (Cueva-Rolón et al. 1993); this might reflect that VP activated SPN axons of both sides or SPN axons of either side produced bilateral activation of TS MNs. In three cats, the SPN was stimulated in both sides either separately or at the same time. SPN stimulation in either side produced similar TS response in the left side. If SPNs of both sides were stimulated at the same time, the contraction of the left TS muscles was larger than the linear summation of the contractions that were produced by separate stimulation of each nerve (Fig. 2E). This result strongly suggests that SPN axons from one side produce similar effects on TS MNs of both sides.

In five cats, the tension increased and decreased in steps separated by plateaus lasting a few seconds (Fig. 3, A–C; see also Fig. 2, B and D), suggesting that a fixed number of active units fired at constant frequency. The smaller tension steps in the downward direction appear to be produced by the sudden firing offset in individual motor units (Fig. 3B). Note in Fig. 3B that stimulating a single motor axon of the soleus muscle at 20 Hz produced a tension plateau of similar amplitude as that of the smaller steps. Larger steps (Fig. 3, A and B) appear to be produced by the synchronous firing onset or offset of several motor units. In four cats, a single shock (1.5 T) to SPN produced visible contraction of a small portion of the gastrocnemius muscle, as well as a small but prolonged step-like tension change; an EMG electrode was inserted in the contracting portion. A single motor unit apparently produced the tension change that is illustrated in Fig. 3C. Note the long latency between the stimulus and the response. In three cats, two motor units from different TS muscles [MG and lateral gastrocnemius or (LG)] were recorded at the same time during a postdischarge. The firing of both units ceased rather suddenly
but at different times (Fig. 3, D). The descending tension phase may show small steps (B). Note in B the sustained contraction of a motor unit in response to stimulation (23 shocks, 30 Hz) of a single TS axon, which was found in a fine filament of the L7 ventral root; the single axon spike was identified by stimulation of the TS nerve. Note also that the amplitudes of the descending steps and of the contraction evoked by stimulation of the single axon are similar. C. SPN was stimulated with a single shock; the activity of a single motor unit of the MG muscle (top) and the TS tension change (middle) were recorded; note that the amplitude of the tension change is similar to that produced by stimulation of a single motor axon (B). Top and middle traces in D–F were taken from the MG and LG muscles; 1 motor unit was recorded in each muscle; the time difference between panels is ~5 s. A single train of shocks was applied to SPN (D); note that the motor units firing started suddenly at the same time and ended also suddenly but at different times.

What causes the prolonged TS contraction?

The sustained firing of TS motor units in nonparalyzed cats (Fig. 3, C–F) could be produced by sustained firing of Ints (Hultborn and Wigström 1980) or by an intrinsic MN property (bistable behavior) (Hounsgaard et al. 1984, 1988). An important part of our project was to decide between these two possibilities.

Tapping on the process of the calcaneum produced a reflex TS twitch (Fig. 4A) (Cueva-Rolón et al. 1993). Below a critical tap force, no twitch was produced (Fig. 4B). The left SPN was stimulated with four to six shocks 1.3 T at 1 Hz; a small TS response outlasting the last shock for 1–4 s was produced (Fig. 4C). If the subthreshold tap was preceded by the SPN stimulation, then the tap triggered a large TS contraction that lasted >30 s (3 experiments; Fig. 4D).

Summation of excitatory postsynaptic potentials (EPSPs) elicited in TS MNs by both spindle afferents and Ints in the SPN pathway could account for the large, fast rising TS contraction. The long duration of the response, however, most likely results from MN bistable firing.

It could be argued that the long duration might result from summation of EPSPs generated by sustained firing of Ints. On one side, SPN stimulation caused a short-lasting (<5 s) MNs postdischarge (Fig. 5C); Ints, however, might have depolarized MNs below the threshold during a longer period. Although unlikely, on the other side, the weak tendon tap might produce disynaptic MNs activation (see Angel et al. 1996; Edgley and Jankowska 1987; McCrea et al. 1995); in theory, Ints in this disynaptic pathway might also in a sustained manner and produce subthreshold MN depolarization, which could add to that produced by SPN stimulation and then reach the MN threshold. Thus MN firing would follow the sustained Ints firing. This possibility cannot be excluded.

Intracellular recordings

The sustained firing of TS motor units in response to VP (Cueva-Rolón et al. 1993) or to SPN stimulation (Fig. 3, C and D–F) suggests that the corresponding MNs were in the active phase of bistable behavior (MN BB); this can be only shown by means of intracellular recordings (Hounsgaard et al. 1988; Hultborn 1999; Lee and Heckman 1998a,b). The cats showed violent movements during and after SPN stimulation. Therefore stable and reliable MN recordings could not be gained in the nonparalyzed cat. In 18 cats that were paralyzed by an injection of pancuronium (Panc), 112 MNs from different pools were impaled; MNs with resting membrane potential (RMP) <55 mV were rejected. 74 MNs were accepted, and 70 of these responded to SPN stimulation; the type of response was not related to the type of MN. Nine MNs responding to SPN did not respond with monosynaptic EPSP to stimulation of the dissected muscular nerves (unidentified MNs; see Table 1). The average RMP and action potentials of the selected MNs.
were, respectively, 62 ± 6 and 76 ± 5.7 mV; the input resistance was 1.6 ± 0.3 MΩ (n = 12), and the axonal conduction velocity was 92 ± 10.1 m/s. The MNs response to single shocks (1.5–2.6 T) to SPN frequently started (n = 40) with an IPSP followed by small EPSP (4.5 mV). Only EPSPs were seen in 12 MNs, and in some cases, no clear response was detected (Fig. 6B).

When the MN showed an IPSP-EPSP sequence at RMP, the IPSP lasted ~10 ms (Fig. 5A). The subsequent, small EPSP declined slowly. The IPSP-EPSP sequence was not seen if current pulses of certain amplitude were applied. When a depolarizing (D) pulse was applied, SPN stimulation produced a larger IPSP that was prolonged >30 ms; that is, at RMP, a late IPSP was masked by the EPSP, but it appeared as the

FIG. 4. Effect of a conditioning stimulus to SPN on the monosynaptic TS reflex. A: reflex twitch tension (7 superimposed records) that was produced by tapping on the calcaneum; the artifact in the bottom trace is the electric pulse that activated the tapping device. B: a weak, below threshold tap was applied to the calcaneum. C: SPN was stimulated with pulses at 2/s; a small TS tension change was produced. D: a tap of the same intensity as in C was applied after a SPN stimulus as in B. See the text for further details.

FIG. 5. Motoneuron (MN) responses to stimulation of SPN with single shock (A; 2.0 T) or with trains of shocks (100 Hz, 400–500 ms; B and C); the bottom trace in each panel is the afferent volley. A and B were taken from the same TS MN. C was obtained from a Tib MN. The 1st synaptic potential is an inhibitory postsynaptic potential (IPSP) in A and B, and an excitatory postsynaptic potential (EPSP) in C. A: top superimposed traces show the current pulses (I) that were applied to change the MN RMP; SPN was stimulated at resting membrane potential (RMP) and during voltage pulses. At RMP, an IPSP-EPSP sequence was seen; when the cell was depolarized, the MN only showed hyperpolarization with respect to the pulse alone; when the MN was hyperpolarized, only depolarization with respect to the pulse was seen. Note in B that during the train, the depolarization (LD) peaks and decays later but grows and reaches maximal amplitude after the end of the train. C: no IPSP was produced, and LD reached maximal amplitude during the stimulus (2.0 T). In both B and C, the membrane potential recovered its initial level after LD. See the text for further details.
driving force increased. The voltage pulses do not change the duration of the conductance change associated to the IPSP. Therefore IPSP shunts the MN membrane. The initial IPSP reversed when a hyperpolarizing (H) pulse was applied; the reversed IPSP was followed by a >30-ms-long depolarization with respect to the H pulse alone. This depolarization, which results from both, reversed IPSPs and the EPSPs, lasted for as long as the IPSP that was evoked when the D pulse was applied. It is concluded that after the initial IPSP, SPN stimulation evokes both EPSPs and IPSPs at the same time. This is important to interpret the MN response when SPN was stimulated with a train of pulses at 100 Hz (Fig. 5).

| TABLE 1. Lumbar motoneurons showing or not showing bistable firing to SPN stimulation |
|-----------------------------------|---|---|---|---|---|
|                                   | pBSt | Tib | TS | DPer | Non-identified | Total |
| Bistable                          | 9    | 5   | 6  | 3    | 7              | 30    |
| Non-bistable                      | 5    | 9   | 3  | 2    | 5              | 24    |

pBSt, posterior biceps and semitendinous; Tib, tibial; TS, triceps surae; DPer, deep peroneal.

The firing frequency was transiently decreased by an IPSP that was evoked by Tib nerve stimulation but it was later increased by subsequent EPSP (Fig. 6E). After the current injection was removed, the MN repolarized in a few seconds (Fig. 6F). This suggests either the persistence of an inward current or a reduction of an outward current. In all MNs that showed sustained firing, a residual depolarization lasting >1 s was seen (Paroschy and Shetchehy 2000).

In 15 MNs, sustained firing in response to SPN stimulation was produced using current pulses that were below the threshold when applied alone (Fig. 7); this firing ensued when the MN was depolarized 2.7 ± 0.3 mV below the threshold. In the case that is illustrated in Fig. 7, the SPN stimulus produced MN postdischarge when the voltage pulse was 17.5 mV more depolarized than the RMP (Fig. 7B). In three motoneurons, a large (∼30 mV), sudden depolarization occurred during the sustained firing (see Fig. 7E, right); a similar depolarization was found in cat MNs (Bennet et al. 1998) as well as in dorsal horn neurons of the spinal cord of the turtle and of the rat (Morisset and Nagy 1998; Russo and Hounsgaard 1996).
Effect of pancuronium

In paralyzed cats, the lack of MNBB at RMP could reflect that all MNs were hyperpolarized after the injection of Panc. It could not be discarded, however, that MNs with MP $\geq 55$ mV, as those used for intracellular recording, might not show bistable firing neither after nor before the Panc injection; other MNs with lower MP might have produced the muscle response. It has been shown that in cats paralyzed with gallamine triethiodide (Flaxedil), SPN stimulation produced postdischarge in axons from sacral MNs (Paroschy and Shefchyk 2000). To test whether the MNs that we selected might have a resting potential that was too large for bistable firing, the response of motor MG axons to SPN train of stimulation was studied before and after Panc injection. For this purpose, a fine filament of the MG nerve was dissected and sectioned, and ENG recordings were taken from the central stump ($n = 7$); the remaining MG nerve was kept intact.

In three experiments, LS and SS were distinguished in the ENG (see METHODS). Before Panc injection, only SS were detected when the MG EMG was flat, and when the EMG showed activity (LS) appeared. These findings suggest that alpha axons and gamma axons might have produced LS and SS, respectively. Before the Panc injection, SPN stimulation caused postdischarge of both, LS and SS (Fig. 8 A, top left, and B, left); the postdischarge of SS lasted longer (Fig. 8A, top left, and B, left, $\bullet$). The increase in LS and SS frequency could not be determined during the stimulus (100 Hz, 200 ms) and 2–3 s later due to cat movement artifacts. The maximal frequency of both LS and SS was $\sim 30/s$ (Fig. 8 B, left). The LS postdischarge frequency decayed gradually and lasted $\sim 60–90$ s; the
SS postdischarge reached its maximal frequency immediately after the stimulus and remained unchanged for >100–120 s.

Five-second-long samples of the rectified and integrated EMG postdischarge were taken (RIEMG; Fig. 8C). The time relation between RIEMG and the postdischarge frequencies of both, LS and SS, were determined. RIEMG was highly correlated with the LS postdischarge (Fig. 8D); no correlation was found between SS and RIEMG. This supported further that SS were generated by gamma MNs, whereas LS were generated by alpha MNs.

After Panc injection, the LS postdischarge lasted <5 s but the SS postdischarge was prolonged >100 s, although the spike frequency showed a progressive decrease that started immediately after the end of the stimulus (Fig. 8A, top right, and B, right); the basal frequency of both, LS and SS also decreased. Essentially the same results were obtained in two other cats (fine filament recording). When thicker filaments or whole MG nerve were used (n = 4), the persistence of SS spikes in the postdischarge after pancuronium may pass unnoticed although it may revealed by ENG integration. The effect of Panc (80 μg/kg) vanished within 50–90 min.

SPN first activates gamma MNs

LS or SS or both, might initiate the ENG postdischarge. To solve this point, attention was paid to the time relations between the stimulus, the ENG spikes and the EMG (nonparalyzed cats; n = 7). Stimulating SPN with single shocks may produce MG postdischarge if the muscle is stretched just below the stretch needed to produce reflex muscle tone (stretch reflex). No postdischarge was produced in the slack muscle, and when the muscle was stretched 2–3 cm, the background ENG and EMG activities increased to a level that obscured the stimulus effect. Clear results were obtained by stretching the muscle 0.5–1 cm.

In four experiments, only one type of ENG spikes, presumably LS could be distinguished; the amplitude of SS might have been within the background noise level. In these cases, however, single-shock stimulation of SPN evoked a small but clear ENG potential, which was not followed by an EMG potential (Figs. 9A and 10A); this finding also suggests that the ENG potential was produced by gamma MNs. Furthermore, the EMG was depressed during 40–50 ms after the SPN stimulus; later, a progressive increase of both, EMG and ENG followed (Fig. 9A) and vanished within a few seconds. The ENG increase started during the EMG depression; this depression might reflect inhibition of alpha-MNs, and the EMG increase that follows the depression reveals a delayed activation of these MNs. Shocks at 5 Hz were applied later to SPN. The ENG and the EMG activities augmented gradually during the SPN train. After the last shock, however, EMG depression followed. The ENG also decreased but remained augmented with respect to control (Fig. 9B, right). Finally, ENG and EMG postdischarge outlasted the stimulus >60 s. The small ENG potential as well as the ENG augmentation during the EMG

![Fig. 9](http://jn.physiology.org/). Activation of gamma axons by stimulation of SPN. Each trace shows 20 superimposed sweeps. ENG was taken from a sectioned MG nerve filament; the EMG was taken from the MG muscle; bottom trace: the stimulus artifact. A: single shock stimulation (1.5 T); note that the ENG spikes are not followed by EMG potential but by EMG depression; during this depression, the ENG showed a mild increase. B, left: control activities; right: recordings taken during and after the last 3 pulses of a series of 25 pulses (5 Hz); the EMG afterdischarge initiates 0.5–1 s after the last pulse; between the last pulse and the postdischarge onset there was a phase of reduced EMG even though the ENG increased with respect to the control (left).
depression might reflect activation of gamma MNs; the subsequent EMG increase reflects facilitation of alpha MNs.

LS and SS were seen in three other experiments; similar results were found in these experiments. If no tension was applied to TS, the EMG was flat or it only showed occasional motor unit spikes; then the ENG mainly or exclusively showed SS. In the experiment that is illustrated in Figs. 8 and 10, the average control frequency was 0.1/s for LS and 2.6/s (SD) for SS. A single shock to SPN produced a series of small ENG spikes that were not followed by EMG potential (Fig. 10A); the average amplitude of these spikes was as small as the one of SS elicited by train stimulation of SPN (compare Fig. 10, A’ and D’). In contrast, a brief stretch to the Achilles tendon produced a reflex EMG response (Fig. 10B), which was preceded by one or two ENG spikes that could change in amplitude from trial to trial, suggesting that different alpha MNs were excited. These spikes were always larger than SS but were not significantly different from those LS that were elicited by sustained muscle stretch or by train stimulation of SPN (compare Fig. 10, B’ and C’).

An analysis similar to that illustrated in Fig. 10 was performed using the data from another cat; the results were comparable.

**DISCUSSION**

**General considerations**

Stimulation of SPN reproduces the motor effects of vaginal probing, and the sustained alpha MN firing reported here is a case of bistable behavior of MNs (BBMs) (see, for example, Crone et al. 1988; Hounsgaard and Kiehn 1985; Hounsgaard et al. 1984, 1988; Hultborn 1999; Lee and Heckman 1998a,b; Morisset and Nagy 1998). MN BB might occur during the mating behavior. The response triggered by stimulation of SPN, however, is not sex-linked. Hind limb extension was also produced in the male cat by SPN stimulation (unpublished

**FIG. 10.** Data obtained from the same experiment as in Fig. 8. The histograms at the right (A’–D’) are amplitude distributions in arbitrary units of the corresponding MG ENG spikes in A–D. A and B: 20 superimposed sweeps of reflex responses to single shock stimulation of SPN (A: 1.5 T) and to brief muscle stretch (B); insets: individual samples; the EMG, which was also recorded in A and B, did not show activity before the stimulus; same amplifications in both, A and B. Note in A that SS spikes were not followed by EMG potential (A). In B, EMG potential followed LS. The histograms in A’ and B’ are for the spikes in A and B, respectively. The spikes in C were recorded during the postdischarge that is illustrated in Fig. 8A (top left); the histogram in C’ is for LS in C (see METHODS). SS in D were recorded during the same postdischarge after LS firing ended. Significant differences were not found between A’ and D’ or between B’ and C’ (P > 0.05). Significant difference (P < 0.001) was found between A’ and B’ as well as between C’ and D’. Both, t-test and Kolmogorov-Smirnov statistic were used.
observations). Nonetheless, the response to VP may be useful to maintain the mating posture (see Eken and Kiehn 1989).

In the female cat, SPN conveys axons from the pudendal skin, the external genitalia, and the vaginal tract (Cueva-Rolón et al. 1994). SPN stimulation triggers a similar TS muscle response in both hind limbs. Thus the rules of reciprocal innervation of hind limb MNs do not appear to apply to afferent fibers from these medial structures.

Excitatory as well as inhibitory Ints were activated by SPN stimulation. The EMG depression-facilitation sequence that was produced by single shocks may reflect an IPSP-EPSp sequence. Because LD outlasted the stimulus for a few seconds, it is likely that it was due to persistent Ints firing.

Identification of SS

Whether we really have identified correctly that gamma MNs produced SS is crucial; four facts support this possibility. First, stimulating SPN with single shocks did not produce alpha-MNs firing but evoked SS in motor axons. Second, an SS-locked muscular potential was not produced. Third, SS and EMG postdischarges were not correlated. Fourth, SS were present when EMG was flat in nonparalyzed cats. In contrast, EMG potential followed the large ENG spikes (LS) that were evoked by tapping on the calcaneum; neither LS nor EMG potential were evoked by single shock stimulation of SPN; LS were absent when EMG was flat but appeared when EMG was activated; and the LS postdischarge frequency was highly correlated with intensity of the EMG postdischarge. LS and SS can be only attributed to the firing of alpha MNs and gamma MNs, respectively.

SPN-gamma-alpha link. The effect of Panc

Gamma MNs can be activated by stimulation of hind limb afferents (Appelberg et al. 1982, 1983). Single shocks to SPN induced stimulus-locked firing of gamma axons; 30–50 ms later, EMG gradually increased at least for 1 s with respect to control. It might be that the gamma firing triggered the delayed alpha firing through an increase in the activity of spindles afferents. Stimulation of SPN with pulse trains caused sustained firing of gamma axons; this might produce a sustained increase in the excitatory inflow from spindle afferents to alpha MNs, which could be depolarized to an adequate level to sustain bistable firing. Drugs that block nerve to muscle transmission do not only act on the extrafusal muscle fibers but they do also act on intrafusal ones (Bessou et al. 1965; Carli et al. 1967; Eyzaguirre 1960; Granit et al. 1953; Hunt 1952; Katz 1949; Yamamoto et al. 1994). A spindle blockade caused by Panc might have decreased the firing of spindle afferents; consequently, the MNs membrane potential would increase.

Sustained firing of gamma axons might result from bistable behavior of the corresponding MNs or from increased activity in a positive feedback loop or from both; the loop might be: gamma MNs-spindle afferents-gamma MNs. The loop firing would decrease after Panc injection. Note that the SS postdischarge decreased faster after Panc with respect to control (see Fig. 8, A and B). The persistence of SS spikes in the postdischarge after LS disappear might be explained by a larger synaptic efficacy in gamma MNs as compared with alpha MNs (size principle) (Henneman et al. 1965).

The effect of Panc warns about trying to produce MN BB at RMP if neuromuscular blocking agents are used. In paralyzed cats (Flaxedil), sacral MNs (Onuf’s nucleus) showed postdischarge lasting <5 s in response to SPN stimulation, but the postdischarge of ENG spikes lasted up to >50 s (Paroschy and Shefczyk 2000). We wonder whether the ENG spikes were generated by gamma axons or whether the alpha MNs of the Onuf’s nucleus are less affected by the spindle paralysis than the alpha MNs of the hind limb. In fact, the EPSP evoked in the former are much larger than those found in the present work (Fedirchuk et al. 1992).

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