

Spinal and Supraspinal Effects of Long-Term Stimulation of Sensorimotor Cortex in Rats

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Chen XY, Pillai S, Chen Y, Wang Y, Chen L, Carp JS, Wolpaw JR. Spinal and supraspinal effects of long-term stimulation of sensorimotor cortex in rats. *J Neurophysiol* 98: 878–887, 2007. First published May 23, 2007; doi:10.1152/jn.00283.2007. Sensorimotor cortex (SMC) modifies spinal cord reflex function throughout life and is essential for operant conditioning of the H-reflex. To further explore this long-term SMC influence over spinal cord function and its possible clinical uses, we assessed the effect of long-term SMC stimulation on the soleus H-reflex. In freely moving rats, the soleus H-reflex was measured 24 h/day for 12 wk. The soleus background EMG and M response associated with H-reflex elicitation were kept stable throughout. SMC stimulation was delivered in a 20-day-on/20-day-off/20-day-on protocol in which a train of biphasic 1-ms pulses at 25 Hz for 1 s was delivered every 10 s for the on-days. The SMC stimulus was automatically adjusted to maintain a constant descending volley. H-reflex size gradually increased during the 20 on-days, stayed high during the 20 off-days, and rose further during the next 20 on-days. In addition, the SMC stimulus needed to maintain a stable descending volley rose steadily over days. It fell during the 20 off-days and rose again when stimulation resumed. These results suggest that SMC stimulation, like H-reflex operant conditioning, induces activity-dependent plasticity in both the brain and the spinal cord and that the plasticity responsible for the H-reflex increase persists longer after the end of SMC stimulation than that underlying the change in the SMC response to stimulation.

INTRODUCTION

The role of sensorimotor cortex in movement initiation and control is well recognized and widely studied (e.g., Ashe et al. 2006; Graziano et al. 2002; Porter and Lemon 1993; Rizzolatti and Luppino 2001). In contrast, its role in the long-term regulation of spinal cord reflex function has received little attention. Descending activity gradually modifies the spinal cord throughout life (reviewed in Wolpaw and Tennissen 2001). However, the pathways and processes through which descending activity induces and maintains spinal cord reflex patterns so that they support effective motor control remain largely obscure. In recent years, new possibilities for restoring function impaired by spinal cord injury, stroke, or other disorders have drawn attention to the mechanisms through which the brain gradually shapes spinal cord reflexes to function properly during movement. Understanding these mechanisms could lead to new techniques for inducing, guiding, and evaluating recovery after injury.

The H-reflex, the electrical analog of the spinal stretch reflex (SSR), is the simplest behavior of the vertebrate CNS. It is

mediated by a wholly spinal and largely monosynaptic pathway, consisting of the primary afferent neuron, the alpha motoneuron, and the synapse between them (Brown 1984; Matthews 1972). Monkeys, humans, rats, and mice can gradually increase or decrease the H-reflex or the SSR when exposed to an operant conditioning paradigm in which reward depends on reflex amplitude (Carp et al. 2006; Chen and Wolpaw 1995; Evatt et al. 1989; Wolpaw 1987; Wolpaw et al. 1983a). By a standard definition of “skill” as an adaptive behavior acquired through practice (Compact OED 1993), these larger or smaller H-reflexes are simple motor skills. The acquisition of these skills occurs over days and weeks and involves plasticity in spinal cord motoneurons, in the synaptic terminals on them, and probably in spinal interneurons and in the brain as well (for review, see Wolpaw 2006; Wolpaw and Tennissen 2001). This spinal cord plasticity appears comparable to that occurring during normal development and skill acquisition and in response to trauma and disease. Furthermore, recent studies indicate that sensorimotor cortex (SMC) activity that descends in the corticospinal tract (CST) is responsible for H-reflex conditioning (Chen and Wolpaw 2002; Chen et al. 2002, 2006a). Thus the H-reflex provides a simple model for exploring long-term SMC control over spinal cord function.

The present study used direct electrical stimulation of SMC, rather than the operant conditioning protocol, to change descending CST activity, and assessed the long-term effects of this stimulation on soleus H-reflexes in freely moving rats. The results suggest that SMC stimulation produces plasticity in the spinal cord and in the cortex as well.

METHODS

Subjects were 11 young (<5 mo old, weight range 410–563 g) male Sprague–Dawley rats. All procedures satisfied the “Guide for the Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC, 1996) and had been reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. The protocol for long-term monitoring of the H-reflex in freely moving rats, fully described previously (e.g., Chen and Wolpaw 1995), is briefly summarized here. The procedures for SMC stimulation and histological analysis are described in detail.

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Implantation of chronic recording and stimulating electrodes

Under general anesthesia (sodium pentobarbital 60 mg/kg, administered intraperitoneally), rats were each implanted with recording or stimulating electrodes in both soleus muscles, on the dorsal surface of the spinal cord at T12, over the SMC areas of both sides, and on both posterior tibial nerves.

To place the stimulating electrodes over the right and left hindlimb areas of SMC, the rat was placed in a stereotaxic frame, with its head leveled and secured by ear bars and a tooth holder (Chen et al. 2006a). Two pairs of stimulation electrodes (stainless steel screws, diameter 0.5 mm) were placed in the skull just above the dura, one pair over each SMC. In each pair, one screw was placed 1.0 mm caudal to bregma and 2.8 mm lateral to the midline and the other was placed 3.0 mm caudal to bregma and 2.8 mm lateral to the midline (Paxinos and Watson 1986).

To elicit the right and left soleus H-reflexes, the right and left posterior tibial nerves were each encircled just above the triceps surae branches with a silicone rubber nerve cuff containing a pair of multistranded stainless steel fine-wire electrodes. To record soleus electromyographic (EMG) activity, the right and left soleus muscles were each implanted with a pair of multistranded stainless steel fine-wire EMG recording electrodes with the final 0.5 cm stripped.

To record the descending spinal cord volley produced by SMC stimulation, a pair of multistranded stainless steel fine-wire electrodes (with 1-mm exposed at the tip and 3-mm separation between the tips) was slipped in above the dura over the dorsal midline of the spinal cord at T12 and secured in place by bone wax and by sutures in the nearby muscle and connective tissue. The Teflon-coated wires from the EMG, nerve cuff, SMC, and spinal cord electrodes passed subcutaneously to a connector plug secured on the skull with stainless steel screws and dental cement.

Immediately after surgery, the rat was placed under a heating lamp and given an analgesic (Demerol, 0.2 mg, administered intramuscularly). Once awake, it received a second dose of analgesic and was returned to its cage and provided with food and water ad libitum. Body weight was measured daily and a high-calorie dietary supplement (Nutri-Cal; 2–4 ml/day, administered per os) was given until body weight regained its presurgery level. Each rat also received a piece of apple (~10 g) every day throughout the study.

H-reflex measurement

Data collection began after the rats recovered from surgical anesthesia and continued for ≤ 4 mo. During this period, each rat lived in a standard rat cage with a 40-cm flexible cable attached to the skull plug. The cable, which allowed the rat to move freely about the cage, carried the wires from the electrodes to an electrical commutator above the cage, and from there to EMG amplifiers (gain 1,000, bandwidth 100–1,000 Hz) and stimulus isolation units. All rats had free access to water and food. Animal well-being was carefully checked several times each day and body weight was measured weekly. Laboratory lights were dimmed from 2100 to 0600 each day.

H-reflex elicitation began ≥ 10 days after the rat had fully recovered from surgery and resumed normal activity (which usually took 3–5 days). Stimulus delivery and data collection

were under the control of a computer system, which monitored ongoing soleus EMG (filtered at 100–1,000 Hz, sampled at 5,000 Hz) in both legs continuously 24 h/day, 7 days/wk, for the entire period of the experiment. Whenever the absolute value (equivalent to the full-wave rectified value) of background (i.e., ongoing) EMG from both the right and left soleus muscles remained within defined ranges for a randomly varying 2.3- to 2.7-s period, the computer initiated a trial. [Because the number of trials/day was inversely related to the stringency of the background EMG criteria, and because the data from the right (i.e., contralateral) soleus were of primary interest, the criteria applied to the left soleus were less stringent. Consequently, the day-to-day variability in background EMG and in H-reflex and M-response sizes tended to be greater for the left soleus.] In each trial, the computer stored the most recent 50 ms of EMG from both muscles (i.e., the background EMG interval), delivered a monophasic stimulus pulse to each nerve cuff, and stored the EMG for another 100 ms. Pulse amplitude and duration were initially set for each side to produce a maximum soleus H-reflex (as well as an M response that was typically just above threshold). Pulse duration (usually 0.5 ms) remained fixed. Pulse amplitude in each leg was adjusted by the computer after each trial to maintain the soleus M response [i.e., average amplitude of EMG in the M-response interval (typically 2.0–4.5 ms after stimulation)] unchanged throughout data collection. This ensured that the effective strength of the nerve stimulus was stable throughout the experiment despite any changes that occurred in nerve cuff electrode impedances or in other factors (Chen and Wolpaw 1995; Wolpaw 1987). H-reflex size was defined as the average amplitude of EMG in the H-reflex interval (typically 6–10 ms after stimulation) minus average background EMG amplitude.

In the course of its normal activity, the animal usually satisfied the background EMG requirement, and thus received nerve cuff stimulation, 2,000–6,000 times per day. For each rat, soleus H-reflex data collection continued for ≥ 12 wk (except for one rat in which data were collected for only 7 wk because of the loss of EMG electrode function). For the first 10–20 days (which served as a control period), no SMC stimulation was delivered. After this control period, SMC stimulation began as subsequently described and H-reflex data collection continued.

SMC stimulation

After the control period, each rat was exposed to a 20-day-on/20-day-off/20-day-on (20/20/20) SMC stimulation protocol. SMC stimulus delivery and measurement of the spinal cord volley response and the right soleus EMG response were controlled by a unit of the computer system separate from the unit that controlled H-reflex elicitation and measurement. In each rat, only the left SMC was stimulated. The stimulation consisted of a 1-s train of 25 1-ms biphasic pulses delivered through the left SMC electrode pair (i.e., 25-Hz pulse rate) every 10 s throughout every other 20 days for 60 days (i.e., 20-on/20-off/20-on, or 20/20/20). Figure 1A shows the stimulation protocol.

The SMC stimulation amplitude was initially set to produce a small spinal cord volley response at the T12 epidural electrodes (earliest component typically 3.5–5.5 ms after stimulus onset) and a small EMG response from the right soleus muscle

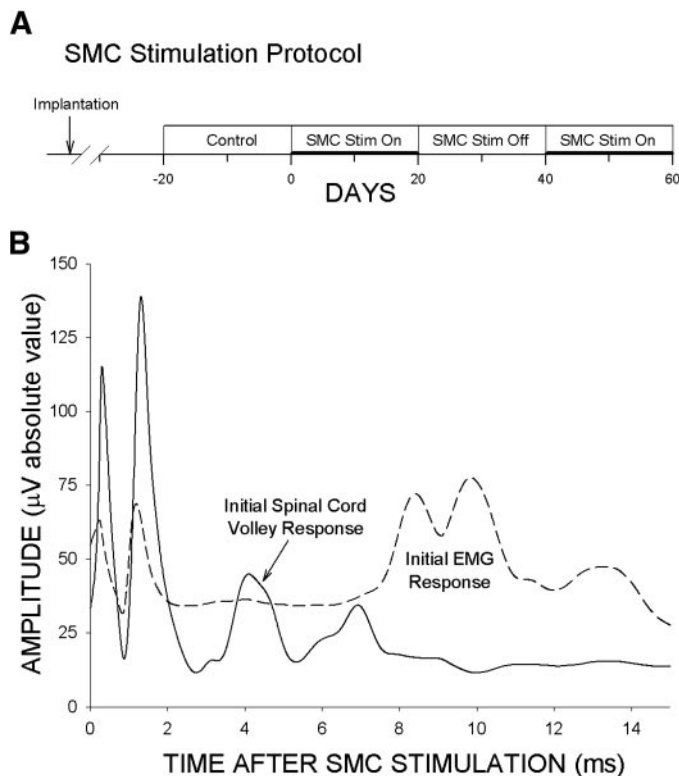


FIG. 1. *A*: sensorimotor cortex (SMC) stimulation protocol. After control-mode data collection, pulse-train SMC stimulation (25 1-ms biphasic pulses at 25 Hz every 10 s) was delivered to the left SMC in a 20-day-on (black bars), 20-day-off alternation. H-reflex collection continued throughout. *B*: average rectified activity for 1 day over the dorsal spinal cord at T12 (solid) and from the right soleus muscle (dashed) after SMC stimulation. SMC stimulus amplitude was automatically adjusted to keep the first component of the spinal cord volley response [or of the right soleus electromyographic (EMG) response] unchanged throughout study (see text). Activity in the first 2 ms is rectified stimulus artifact.

(typically 7.5–11.0 ms after stimulus onset). The epidural and EMG signals were filtered at 100–1,000 Hz and their absolute value was sampled at 5,000 Hz. The computer calculated the T12 epidural response and the EMG response to the first pulse of the train. Figure 1*B* shows typical spinal cord and soleus responses from a rat. From the beginning of SMC stimulation, the stimulus amplitude was automatically adjusted after each stimulation to maintain the earliest component of the spinal cord volley response unchanged throughout data collection. In six of the 11 rats, the spinal cord volley continued to control SMC stimulus amplitude throughout data collection. In these rats, both the spinal cord volley and the right soleus EMG response remained stable throughout. In the other five rats, the spinal cord volley controlled SMC stimulus amplitude for the first 5–15 (11 ± 4 SD) days of each 20-day stimulation period, during which time both the spinal cord volley and the right soleus EMG response were stable. Subsequently in these five rats, the progressive growth of the stimulus artifact [probably arising from the progressive increase in SMC stimulus amplitude (see RESULTS)] obscured the cord volley response and the earliest component of the right soleus EMG response was thenceforth used to control SMC stimulus amplitude. In these five rats, as in the other six, the right soleus EMG response remained stable throughout data collection.

The computer stored the digitized absolute-value data from the T12 epidural and soleus EMG electrode pairs for 50 ms before and 100 ms after onset of the SMC stimulation. As noted earlier, H-reflex data collection continued throughout the 20/20/20 SMC stimulation protocol.

The SMC stimulation protocol functioned well. SMC stimulation caused no apparent distress and produced no visible response. The rats continued to thrive throughout. They remained active and gained weight steadily.

Histology and immunohistochemistry evaluation

At the end of study, each rat was killed with an overdose of sodium pentobarbital and perfused through the heart with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The EMG, nerve cuff, and spinal cord volley electrodes, the T12 segment of spinal cord, the posterior tibial nerves, and the soleus muscles were examined, and the soleus muscles of both sides were removed and weighed. The brain was removed and the cortical areas just below the right and left pairs of screw electrodes were examined. In all rats, the SMC stimulating electrodes were found to be located above the hindlimb SMC area as defined by Paxinos and Watson (1986).

The effect of SMC stimulation on the SMC itself was evaluated in three rats. The neurons and astrocytes of the stimulated (i.e., the left) and the unstimulated (i.e., the right) SMCs were quantified using histological and immunohistochemical methods (Spataro et al. 2005). All three rats were killed 3 days after the end of the second 20-day SMC stimulation period. (The 3 days allowed time for soleus motoneuron labeling by cholera toxin-HRP injected into the muscle for another study.) After perfusion, the brains were removed, dissected, and postfixed in the same fixative (i.e., 4% paraformaldehyde) for 24 h at 4°C. Serial coronal sections (100 μ m thick) were cut through the right and left SMC areas (i.e., the areas below the screws) with a vibratome. The sections were treated with 5% sodium borohydrate (30 min) and 0.2% Triton X-100 in HEPES-buffered Hanks saline (HBHS) (30 min) and incubated overnight with bovine serum albumin (BSA) in HBHS. The next day, the sections were washed with HBHS four times (30 min each) and incubated overnight with monoclonal mouse anti-GFAP (glial fibrillary acidic protein) (1:3,000 dilution, Sigma, St. Louis, MO) with 2.5% horse sera. The following day, after four washes (30 min each) with 0.5% Tween-20 in HBHS, the sections were incubated overnight with Texas Red conjugated goat anti-mouse IgG (1:200 dilution, Molecular Probes, Eugene, OR), Nissl-neuro trace deep red 640/660 (1:100 dilution, Molecular Probes), and CyQuant (1:1,000 dilution, Molecular Probes). Stained sections were washed and mounted between coverslips in Gel/Mount aqueous mounting media (Biomedica, Foster City, CA). Immunohistochemistry controls that were processed without primary antibody for GFAP, Nissl stain, or CyQuant stain were negative for staining for that antibody.

Confocal microscopy and image collection followed Spataro et al. (2005). Briefly, images were collected as three-dimensional (3-D) data sets with a $\times 20$ objective lens on an Olympus IX 70 inverted fluorescence microscope with a NORAN confocal laser scanning attachment (NORAN Instruments). Samples from both the left (stimulated) and right (unstimulated control) SMC areas of each section were imaged through the

entire thickness of the section. The focused X/Y image projections were stacked through the entire thickness (i.e., the Z-dimension) of the section (Intervision Software, NORAN Instruments) to provide a composite focused image of the entire section.

For each SMC area of each rat, three to five composite images of 100- μm -thick sections located 400–600 μm apart were fully analyzed. To assess neuron number in each composite image, the neurons in a 300- μm (horizontal) \times 150- μm (deep) area beginning 125 μm below the pia of the right and left SMC areas were automatically counted using the 3D-CATFISH cell-counting program (Lin et al. 2003). To assess astrocyte number in each composite image, the astrocytes in a 300- μm (horizontal) \times 260- μm (deep) area just below the pia mater were counted by two independent raters who did not know whether the image came from a stimulated (i.e., left) or an unstimulated (i.e., right) SMC. This area was divided into two 300 \times 130- μm areas (one superficial and one deep) and the average number of astrocytes in each region was determined.

Data analysis

Background EMG amplitude was calculated as average EMG amplitude during the 50 ms before nerve stimulation. M-response size was calculated as average EMG amplitude in the M-response interval (typically 2.0–4.5 ms after the tibial nerve stimulation) minus average background EMG amplitude. H-reflex size was calculated as average EMG amplitude in the H-reflex interval (typically 6.0–10.0 ms after the nerve stimulation) minus average background EMG amplitude. For each leg of each rat, daily averages of soleus background EMG, M-response, and H-reflex values were calculated and expressed in percentage of their average initial values. Each rat's initial background EMG, M-response, and H-reflex values were the average values for the 10 days immediately before SMC stimulation began. The average daily amplitude of the SMC stimulus was calculated and expressed in percentage of the average daily amplitude for the first day of SMC stimulation.

In all rats, background EMG and M-response size remained stable throughout data collection. To assess the effect of SMC stimulation on the H-reflex, a paired *t*-test was used to compare average daily H-reflex sizes for a defined period to initial H-reflex sizes. In addition, the average daily H-reflexes were used to calculate the average course of H-reflex size throughout data collection. To assess the effect of electrical stimulation on SMC, a nested ANOVA (with each measure nested within rats) was used to compare numbers of neurons and numbers of astrocytes in the stimulated (left) and unstimulated (right) SMC areas.

RESULTS

All rats remained healthy and active and continued to gain weight throughout the study. Body weight increased from 495 ± 47 g (mean \pm SD) (range 410–563 g) at the time of implantation surgery to 598 ± 60 g (range 518–706 g) at the time of perfusion. Soleus weights averaged 0.26 ± 0.05 g (mean \pm SD) on the right and exactly the same on the left, and did not differ significantly (measured in percentage of body

weight) from those of 113 normal rats previously studied ($P > 0.48$ for both right and left) (e.g., Chen and Wolpaw 1995, 2002; Chen et al. 2001, 2002, 2003, 2006a). In all rats, the EMG electrodes, the nerve cuffs, the T12 spinal cord epidural electrodes, and the cranial screw electrodes were located where they had been implanted. The nerve cuffs were covered by connective tissue and the tibial nerves of both legs were well preserved inside the cuffs. That the tibial nerves of both sides remained intact structurally and functionally was further indicated by the normal muscle weights and their bilateral symmetry, and also by the fact that the average daily amplitude of the nerve cuff stimulus needed to elicit the target M response remained stable throughout data collection. The spinal cord appeared to be in good condition and did not show any gross morphological differences from that of a normal rat. The meninges and cortex under the screws on both the stimulated (left) and unstimulated (right) sides appeared normal and similar to adjacent areas not under the screws, except that in some rats the cortical surface under the screw was slightly indented. (See below for histological analysis of cortex.)

All rats had soleus H-reflexes. Initial H-reflex size (i.e., average of final 10 control days before SMC stimulation) averaged 103 ± 16 μV (mean \pm SE) on the right and 89 ± 23 μV on the left. Right and left H-reflex sizes did not differ from each other ($P > 0.6$ by *t*-test) nor from those of 135 normal rats previously studied ($P > 0.9$ by *t*-test) (e.g., Chen and Wolpaw 1995, 2002; Chen et al. 2001, 2002, 2003, 2006a). (In one rat in which right soleus EMG recording was lost shortly after control data collection, and in another in which the left tibial nerve cuff did not function, H-reflexes were studied on only one side.) Previous studies have shown that long-term H-reflex elicitation comparable to that of the present study has in itself no effect on H-reflex size (Chen et al. 2006a).

Effects of SMC stimulation on the H-reflex

As shown in Fig. 1A, the rats were exposed to the 60-day SMC stimulation protocol (i.e., two 20-day stimulus-on periods and the intervening 20-day stimulus-off period) after an initial 10- to 20-day period of control data collection. Figure 2 (*left*) summarizes the effects of the SMC stimulation on the contralateral (i.e., right) soleus H-reflex. It shows the average (\pm SE) daily H-reflex for 10 rats for the final 10 control days and for the 60 days of the 20/20/20 protocol in percentage of the average value for the final 10 control days. The H-reflex increased steadily during the first on-period and then continued to rise more gradually during the off-period and the second on-period. For the final 10 days of the second on-period, the H-reflex averaged 161 ± 7 (mean \pm SE) of its initial value, and was significantly increased ($P < 0.001$ by paired *t*-test) from its initial value. At the same time, as Fig. 2 (*left*) also shows, the background EMG and M response were stable over the 70 days of data collection.

Figure 2 (*right*) summarizes the effect of the 20/20/20 protocol on the ipsilateral (i.e., left) soleus H-reflex. Like the right H-reflex, the left H-reflex increased gradually during the first 20-day SMC stimulation. It changed little during the off-period and then rose further during the second on-period. For the final 10 days of the second on-period, the ipsilateral H-reflex averaged $157 \pm 12\%$ and was significantly increased from its control value ($P = 0.001$ by paired *t*-test). For the

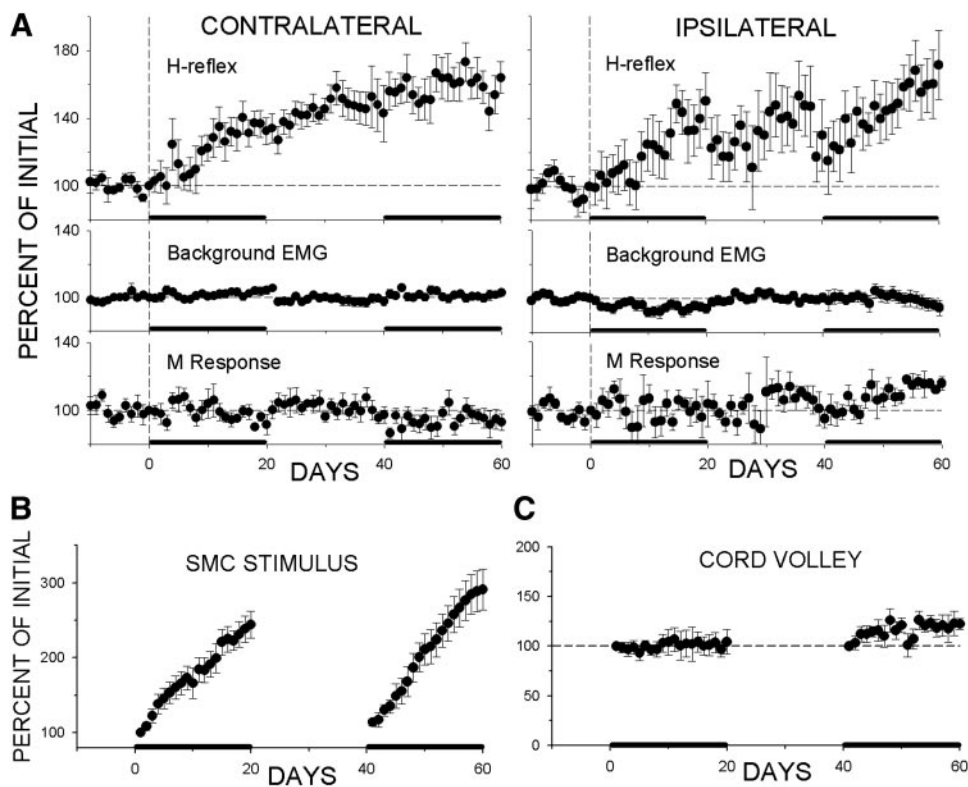


FIG. 2. Average data for all rats exposed to the 20-day-on/20-day-off/20-day-on (20/20/20) stimulation protocol, for the final 10 control days before and for 60 days after the beginning of SMC stimulation. *A*: average daily contralateral (*right*) and ipsilateral (*left*) soleus H-reflex, background EMG, and M response in percentage of average value for the final 10 control days. (Because each rat's data were expressed in units of its average value for the final 10 control days, the values for the control days varied around 100% for every rat. For this reason, and because the H-reflex increase associated with SMC stimulation varied across rats, the average H-reflex values for all rats together show greater variation subsequent to the control period.) *B*: average daily SMC stimulus amplitude in percentage of its value for the first day of stimulation. *C*: average daily cord volley (or right soleus EMG response; see METHODS) in percentage of its value for the first day of stimulation. H-reflex increases gradually after SMC stimulation begins, whereas the M response and background EMG remain stable. SMC stimulus amplitude increases steadily during the first SMC stimulation period, declines almost to its initial level during the 20-day-off period, and then increases again during the second SMC stimulation period, whereas the spinal cord volley (or right soleus EMG response) does not change significantly during either 20-day-on period.

reasons indicated earlier in METHODS, the left soleus background EMG and M response varied more than those of the right soleus M response. The transient decreases in left soleus H-reflex size at the beginning and near the end of the 20-day stimulation-off period may reflect this higher variability. Nevertheless, it is clear that neither the background EMG nor the M response of either side showed a statistically significant change over the 70 days of data collection (see following text).

Effects of SMC stimulation on SMC response to stimulation

SMC stimulation had an additional and unexpected effect: the amplitude of the SMC stimulation pulse needed to maintain a constant spinal cord volley (or a constant right soleus EMG response; see METHODS) rose gradually. Figure 2*B* shows the average (\pm) daily stimulus amplitude (in percentage of the first-day average). It rises steadily and, by Day 20, it is $244 \pm 18\%$ of the pulse for the first on-day. The increase was statistically significant ($P < 0.001$ by paired *t*-test). After the 20-day-off period, stimulus amplitude had fallen almost to its initial value (i.e., to $113 \pm 5\%$) and it then rose steadily again, reaching a value of $297 \pm 27\%$. This increase was also significant ($P < 0.001$, paired *t*-test, for the second 20-day-on period's final day vs. its first day). Linear regression analysis indicated that for both the first and second 20-day-on periods, the pulse amplitude increase was significantly correlated with days of stimulation ($R = 0.99$, $P < 0.001$ for each). The correlations for the two stimulation periods were not significantly different from each other ($P > 0.16$ by *t*-test). Furthermore, the stimulus amplitude at the beginning of the second on-period was higher than that at the beginning of the first on-period ($P = 0.005$, paired *t*-test) and the stimulus amplitude at the end of the second on-period was higher than that at the

end of the first on-period ($P = 0.047$). However, as Fig. 2*C* shows, the spinal cord volley (or soleus EMG response; see METHODS) remained stable throughout the two 20-day periods of SMC stimulation.

Figure 3 shows average contralateral (*right*) and ipsilateral (*left*) H-reflex, background EMG, and M-response data for each 10-day period. To evaluate the effect of the SMC stimulation over the 70 days of data collection, the average values for each 10-day period were analyzed with one-way repeated-measures ANOVA to detect an effect at the $P < 0.05$ level. If an effect was found, the Bonferroni multiple comparisons method was used to determine which poststimulus 10-day period differed significantly from the prestimulus initial values [i.e., average values of final 10 control days (days -10 to 0)]. The SMC stimulation had significant effects on both the right and left soleus H-reflex ($P < 0.001$ for both by repeated-measures ANOVA). The right soleus H-reflex was not different for days 1–10 after stimulation began ($P = 1.0$ by Bonferroni *t*-test) and was significantly increased for all the subsequent 10-day periods [$P = 0.008$ for days 11–20; $P = 0.001$ for days 21–30; and $P < 0.001$ for days 31–40, 41–50, and 51–60 (by Bonferroni *t*-test)]. The left soleus H-reflex was not different for days 1–10 ($P = 1.0$) and days 21–30 ($P = 0.353$), and was significantly increased for all the other 10-day periods ($P = 0.044$ for days 11–20; $P = 0.036$ for days 31–40; $P = 0.004$ for days 41–50; and $P < 0.001$ for days 51–60). The right and left soleus did not differ in final (i.e., days 51–60) H-reflex size ($P = 0.78$, *t*-test). These results indicate that the effect of stimulation on the H-reflex is bilateral, although it appears to be somewhat greater on the contralateral side. In contrast, soleus background EMG and M responses did not change significantly over the 70 days of data collection. One-way repeated-measures ANOVA did not detect any significant

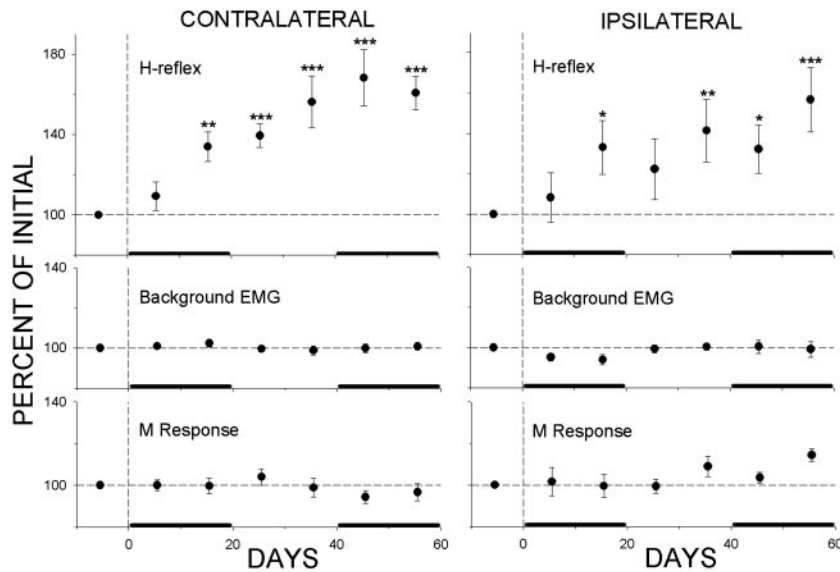


FIG. 3. Average values of contralateral (*right*, *A*) and ipsilateral (*left*, *B*) soleus H-reflex, background EMG, and M response for each 10-day period (in percentage of average value for the final 10 control days) for the final 10 control days and the subsequent 60 days of the 20/20 SMC stimulation protocol. Significant increases in H-reflex size are indicated (***) $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. No significant changes occur in background EMG or M responses.

effect on the right or left soleus background EMG ($P = 0.68$ and $P = 0.06$, respectively), or on the right or left soleus M response ($P = 0.36$ and $P = 0.06$, respectively).

Figure 4 shows average daily contralateral (*right*) and ipsilateral (*left*) soleus peristimulus EMG from a representative rat for a day before SMC stimulation began and for a day at the end of the 20/20 SMC stimulation protocol. Both contralateral and ipsilateral H-reflexes are much larger at the end of the stimulation, whereas the background EMG and M responses remain stable.

Effects of SMC stimulation on SMC histology

For three rats exposed to the 20/20/20 SMC stimulation protocol, we compared the stimulated (i.e., left) and unstimulated (i.e., right) SMCs with respect to numbers of neurons and astrocytes in both superficial and deep areas. Table 1 summarizes the results. The stimulated and unstimulated sides did not differ significantly in number of neurons ($P = 0.99$ by nested ANOVA) nor in number of astrocytes in the superficial or deep areas ($P = 0.07$ and $P = 0.19$ for superficial and deep areas, respectively), although the number of astrocytes in the superficial area was greater on the stimulated side. Figure 5 shows representative confocal images of sections of stimulated and unstimulated SMC areas from one rat. The similarity in neuron number is clear. At the same time, both the number of astrocytes and the GFAP reactivity in the more superficial area are greater on the stimulated side. Thus although SMC stimulation did not produce detectable neuronal loss beneath the electrodes, it may have induced some superficial gliosis.

DISCUSSION

This study was motivated in general by the growing evidence that descending activity shapes spinal cord function during development, throughout later life, and in response to trauma or disease (for review, see Wolpaw and Tennissen 2001), and it was motivated in particular by the evidence that SMC output by the corticospinal tract (CST) induces the spinal cord plasticity that underlies operant conditioning of the H-reflex (Chen and Wolpaw 2002; Chen et al. 2002, 2006a). The

study protocol provided a fixed SMC output for 20 days and evaluated the effect of this stimulation on spinal cord function as reflected in the H-reflex. In an effort to maximize this effect, the SMC stimulus was a pulse train (e.g., Haghghi and Gaines

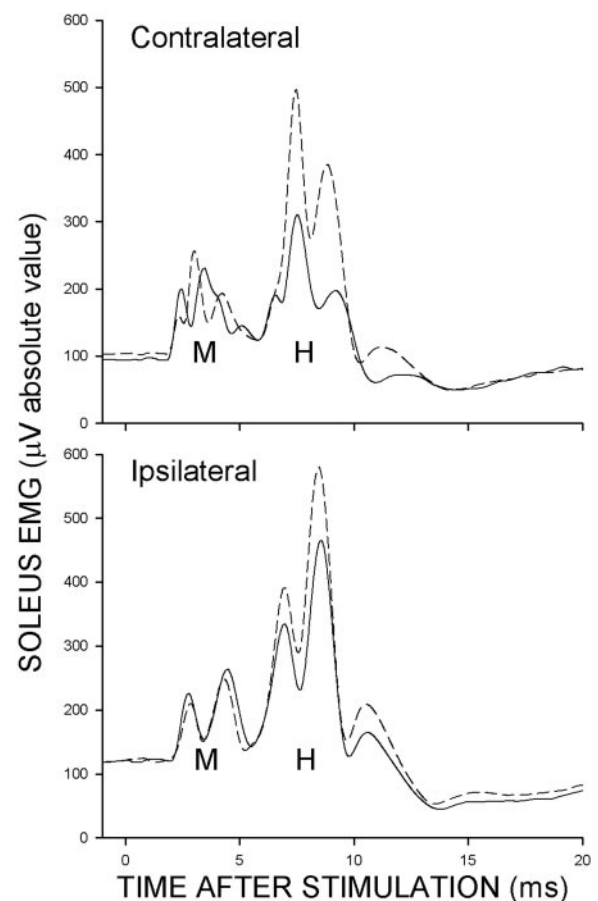


FIG. 4. Average daily contralateral (*right*) and ipsilateral (*left*) peristimulus EMG from a representative rat for 1 day before SMC stimulation began (day -1) (solid lines) and for the last day of the first 20-day stimulation period (i.e., day 20) (dashed lines). On both sides, the H-reflex is larger at the end of stimulation, whereas background EMG (indicated by the EMG level just before the stimulus) and M response remain stable.

TABLE 1. Average values of number of neurons and number of astrocytes in superficial and deep SMC regions for the stimulated and unstimulated SMCs

	Number of Neurons	Number of Astrocytes	
		Superficial	Deep
Stimulated	120 ± 9	31 ± 2	13 ± 1
Unstimulated	121 ± 9	27 ± 2	13 ± 1

Values are average ± SE. Although the number of astrocytes in the superficial area was somewhat greater on the stimulated side, no significant differences were found by nested ANOVA.

2003; Kawaguchi et al. 1996; Meng et al. 2004; Phillips 1969). The results show that SMC stimulation produces gradual and lasting change in H-reflex size and also has an unexpected and surprising effect on SMC itself.

Effects of SMC stimulation on the H-reflex

Twenty days of SMC stimulation gradually increased the H-reflex. The increase was maintained (and may even have continued to grow) for ≥20 days after the stimulus was turned off and grew further during a second 20 days of stimulation. Both contralateral and ipsilateral H-reflexes were affected, although the contralateral effect appeared to be greater. Figures 2A, 3, and 4 summarize and illustrate these results. Throughout the study, the background EMG at the time of H-reflex elicitation remained the same and the M response showed little or no change. Furthermore, previous data show that repetitive elicitation of the H-reflex, like that performed in the present study, does not itself increase H-reflex size (Chen et al. 2006a; unpublished data). It is possible that the H-reflex increase resulted from the combination of SMC stimulation and H-reflex elicitation. However, such effects of combined stimuli typically involve paired presentation (e.g., Stinear and Hornby 2005), which did not occur in the present study.

The plasticity responsible for this long-term effect could be either spinal or supraspinal, or both. Spinal cord plasticity might include one or more of the changes associated with H-reflex conditioning [i.e., changes in motoneuron firing threshold and axonal conduction velocity; in several different synaptic populations on the motoneuron; in the primary afferent–motoneuron synaptic connection; in spinal interneurons (Carp and Wolpaw 1994, 1995; Carp et al. 2001; Feng-Chen and Wolpaw 1996; Wang et al. 2006a; Wolpaw and Chen 2001)]. The possibility that the H-reflex changes produced by SMC stimulation and by operant conditioning depend on related spinal cord plasticity is supported by recent data (Pillai et al. 2006; Wang et al. 2006b), suggesting that SMC stimulation, like H-reflex conditioning (Feng-Chen and Wolpaw 1996; Wang et al. 2006a), affects certain synaptic terminals on the motoneuron, although the relationship between these anatomical effects and the H-reflex changes are not yet clear. On the other hand, the H-reflex increase caused by SMC stimulation might be explained by a mechanism different from those currently implicated in H-reflex operant conditioning. One possibility is a decrease in presynaptic inhibition, which might be mediated by the CST and/or a number of other descending tracts (Rudomin and Schmidt 1999).

The clear importance of the CST to acquisition of H-reflex up-conditioning (Chen et al. 2002) suggests that it might also

be responsible for the long-term H-reflex increase induced by SMC stimulation. H-reflex up-conditioning and the H-reflex increase induced by SMC stimulation are similar in that both persist beyond removal of the descending influence: the increase caused by up-conditioning survived CST transection (Chen et al. 2003) and the increase caused by SMC stimulation lasted through the 20-day-off period.

On the other hand, although both SMC stimulation and H-reflex up-conditioning produce a long-term increase in the soleus H-reflex, they appear to differ in specificity. The increase produced by operant conditioning is focused mainly on the muscle that determines reward (Chen et al. 2005; Wolpaw et al. 1983b, 1993), whereas the increase produced by SMC probably affects the H-reflexes of other ipsilateral muscles, and clearly affects the other (left) soleus nearly as much as it does the right soleus. Furthermore, whereas H-reflex conditioning depends only on the CST (other major descending pathways are not necessary) (Chen and Wolpaw 2002; Chen et al. 2002), it is likely that SMC stimulation affects other cortical and subcortical areas and thereby produces activity in other descending pathways that may contribute to the H-reflex change. Pathways other than the CST are thought to be responsible for H-reflex changes in a variety of situations (e.g., Nielsen and Petersen 1995; Ung et al. 2005). Studies of the impact of specific pathway transections on the effects of SMC stimulation on H-reflex size are needed to clarify this issue.

Supraspinal plasticity might also play a role in the long-term effects of SMC stimulation, as it does in H-reflex operant

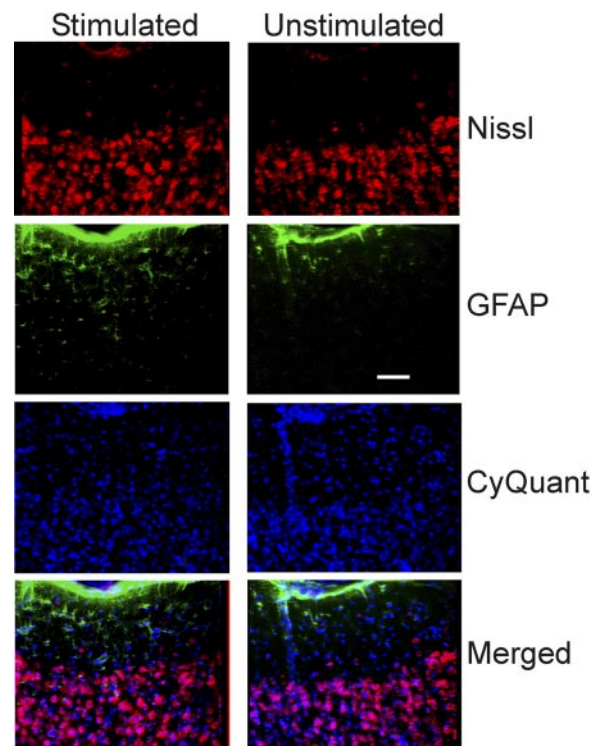


FIG. 5. Confocal images of sections of left (stimulated) and right (unstimulated) SMC areas from a rat exposed to the 20/20/20 left SMC stimulation protocol. Top to bottom: Nissl staining (showing neurons), GFAP (glial fibrillary acidic protein) labeling (reflecting astrocytes), CyQuant staining (showing nuclei), and merged image. Neuronal number is similar on the 2 sides. Greater GFAP labeling and CyQuant staining in the upper regions on the stimulated side suggest modest superficial gliosis. Scale bar is 50 μm for all images.

conditioning (Wolpaw and Chen 2006). As discussed in the next section, cortical stimulation clearly has long-term effects on cortical function (Chen et al. 1997; Peinemann et al. 2004); it also affects function in related areas including contralateral cortex, thalamus, basal ganglia, and cerebellum (Chen et al. 1997; Lang et al. 2004; Peinemann et al. 2004; Sgambato et al. 1997).

In this regard, it is particularly interesting that SMC stimulation typically produced a response in the contralateral soleus and little or no response in the ipsilateral soleus, but nevertheless increased the H-reflex bilaterally. This raises the possibility that spinal cord plasticity responsible for H-reflex increase was produced not by the descending volley elicited by SMC stimulation but rather by descending activity resulting from the supraspinal effects of the SMC stimulation. The evidence that SMC changed SMC itself (discussed in the next section) suggests that it also changed ongoing SMC activity. Such change, if it involved SMC bilaterally, might have led to the bilateral H-reflex increase.

Effects of SMC stimulation on the SMC

The most surprising result of the present study was that the SMC stimulus amplitude needed to maintain a constant spinal cord volley response (or a constant right soleus EMG response; see METHODS) rose steadily as stimulation continued (Fig. 2, B and C). Because the SMC stimulation protocol used a biphasic pulse, it is not likely that this effect was the result of gradual electrode polarization. Histological analysis provided no evidence for substantial stimulation-induced damage to the cortex. Although a modest superficial gliosis probably occurred, no neuronal loss was apparent. The fact that the increase in stimulus amplitude disappeared almost completely over the 20-day-off period also suggests that it was not attributed to cortical damage, but rather reflected stimulation-induced plasticity in SMC.

Electrical stimulation can activate pyramidal tract (i.e., CST) neurons either directly at the axon hillock or indirectly through synaptic input from other neurons (for review, see Iles 2005; Rothwell 1997). The relatively weak stimuli (i.e., 10–30 μ A) used in this study, the high sensitivity of CST neurons to direct stimulation (Tehovnik et al. 2006), and the short latency of the spinal cord volley suggest that CST neurons were activated directly. Short-term stimulation can clearly have acute effects on SMC function (e.g., Jiang et al. 1990). Continued stimulation, as in the present study, might eventually change neuronal properties (e.g., firing threshold) (Carp and Wolpaw 1994; Carp et al. 2001) and/or indirectly affect tonic inputs to CST neurons that affect their response to the stimulation. Such plasticity would constitute compensatory, or homeostatic, plasticity that maintains stable function in spite of long-term changes in input or in other influences (Siebner et al. 2004; Wierenga et al. 2005; Wolpaw and Lee 1989).

SMC stimulation produces plasticity at multiple sites

As discussed in the previous sections, the results show that the prolonged SMC stimulation affected both spinal cord function, as reflected in the H-reflex, and cortical function, as reflected in the stimulus strength needed to produce a given descending volley. Although the sites and nature of the plas-

ticity underlying these functional effects are as yet uncertain, it seems clear that the SMC stimulation produces plasticity of several kinds and that this plasticity is probably spinal as well as supraspinal. The existence of multiple plastic changes is demonstrated most clearly by the contrast between the persistence of the H-reflex change and the almost complete disappearance of the change in stimulus strength over the 20-day stimulation-off period.

Results from a variety of experimental models indicate that multisite plasticity occurs with even the simplest learning phenomena (Blazquez et al. 2002; Carey and Lisberger 2002; Cohen et al. 1997; Hansel et al. 2001; King et al. 2001; Lieb and Frost 1997; Medina et al. 2002; Mendell 1984; Whelan and Pearson 1997; Wolpaw 2002; Wolpaw and Lee 1989; Wolpaw and Tennissen 2001; van Alphen and De Zeeuw 2002). It appears that the behavioral changes produced by these models depend on sequential plasticity and on interactions between plasticity at different sites (e.g., Krakauer and Shadmehr 2006; Wolpaw and Chen 2006). Furthermore, for the CNS to maintain its roster of important behaviors, additional compensatory plasticity may occur to eliminate decrements in previously learned behaviors produced by the plasticity responsible for a new behavior (Wolpaw and Tennissen 2001). Similar processes of sequential, interactive, or compensatory plasticity may underlie the complex functional effects of prolonged SMC stimulation.

Behavioral effects and possible therapeutic uses

A number of studies indicate that SMC stimulation of various kinds not only can affect motor function, but also can improve function in patients after cortical damage arising from stroke (Brown 2006; Hummel and Cohen 2005; Pascual-Leone 2006; Uy et al. 2003). Furthermore, these improvements can persist for prolonged periods after stimulation ends. The present study provides some insight into how spinal reflex changes might contribute to such improvements and suggests how specific improvements might be targeted and achieved in the future.

The H-reflex changes produced by operant conditioning are still evident during locomotion (Chen et al. 2005). Whether this is also true for the H-reflex changes produced by SMC stimulation remains to be determined. A recent study shows that operant conditioning of H-reflex can be used to improve locomotor function after partial spinal cord injury in rats (Chen et al. 2006b). It is possible that the spinal or supraspinal plasticity induced by long-term SMC stimulation might also be used in this way. Stimulus regimens might be selected according to the characteristics and needs of each individual so as to induce more effective spinal cord function. They might become particularly useful once significant regeneration becomes possible (Muir and Steeves 1997; Wolpaw 2006) and techniques for adjusting spinal cord reflex pathways so that they support effective function become essential.

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