INTRODUCTION

The task of scratching movements is to remove an irritating object from the skin. In the cat, the receptive field of the scratch reflex covers the uppermost part of the body, the neck, and the head (Carlson-Kuhta and Smith 1994; Deliagina et al. 1975; Kuhta and Smith 1990; Sherrington 1917). The protraction stage of the scratch response includes bending of spine, hind limb protraction, and turning of the head toward the limb. During the rhythmic stage, the activity of flexor and extensor muscles of the protracted limb alternate and the limb paw rubs rhythmically against the stimulated site. As was emphasized by Sherrington (1910), rhythmic scratching movements are fairly stereotypic, whereas the posture depends on the position of irritant. Experiments on the decerebrate and spinal cats have shown that rhythmic motor pattern similar to scratching can be evoked by electrical or chemical stimulation of the cervical spinal cord; the patterns persisted after the limb deafferentation (Deliagina et al. 1975; Feldberg and Fleischhauer 1960; Sherrington 1917). Similar finding were reported in guinea pigs, frogs, turtles, and dogs (Brown 1909; Fukson et al. 1980; Sherrington 1906; Stein 1988). It was concluded that the basic mechanism of the scratching response is located in the spinal cord. In decerebrate cats, however, it was shown that brain stem mechanisms also contribute to the control of scratching. First, the efferent activity in these preparations possessing some suprasegmental centers differed from that in the spinal cats (Degtyarenko 1990). Second, electrical stimulation of different descending systems—vestibular nucleus, red nucleus, and pyramidal tract—aﬀected the value and the timing of scratching movements (Degtyarenko et al. 1992). Third, the activity of reticulospinal, vestibulospinal, and rubrospinal neurons was modulated in relation to scratching (Arshavsky et al. 1978a,b; Pavlova 1977). The experiments on decerebrated cats precluded testing the participation of the motor cortex in the control of scratching, however.

The goal of this study was to investigate the activity of individual neurons from the hind limb representation of the motor cortex in awake cats during scratching. We examined both pyramidal tract neurons (PTNs) and nonidentified cells (nIDs). We found that the activity of many of these neurons is strongly related to both the protraction and the rhythmic stages of the response. This finding suggests that the motor cortex may participate in the generation and/or modulation of highly stereotypic motor patterns.

A brief account of this study was published in abstract form (Pavlova et al. 2003).

METHODS

Recordings were obtained from the hind limb representation of the motor cortex of three chronically instrumented awake adult cats (1 male and 2 females), which sat in a “sphinx” position in a head restraining device. Some of the methods have been described (Beloozerova and Sirota 1993a; Beloozerova et al. 2003b; Prilutsky et al. 2005) and will be reported briefly here. All experiments were conducted in accordance with National Institutes of Health guidelines and were approved by the Barrow Neurological Institute Animal Care and Use Committee.

Surgical procedures

Surgery was performed under isoflurane anesthesia using aseptic procedures. The skin and fascia were retracted from much of the dorsal surface of the skull. At 10 points around the circumference of the head, stainless steel screws were screwed into the skull and connected together with a copper wire; the screw heads and the wire were inserted into a plastic cast to form a circular base. During recording experiments, the head of awake cats was rigidly held by this base. The base was also used for fixation of connectors, a miniature microdrive, and a protective and electrically shielding cap. The parts were secured to the skull with stainless steel wires.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
of os frontale and os ethmoidale above the sigmoid gyrus on the left side were removed. The region of the motor cortex was visually identified by surface features and photographed. The dura above the motor cortex, over ~0.6 cm², was removed. The aperture was covered by a plastic plate 1 mm thick, in which ~100 holes 0.3 mm in diameter had been drilled and prefilled with sterile wax. The plate was fixed to the surrounding bone by orthodontic resin (Densply Caulk).

Two 26-gauge hypodermic guide tubes were implanted vertically above the medullary pyramid with the tips at the Horsley and Clarke coordinates (P 10; L 0.5) and (P 9.3; L 1.2), at the depth of H 0. Stimulating electrodes were inserted into the medullary pyramid later in the awake cat.

A pair of recording leads constructed from teflon-insulated multi-strand stainless steel wire (AS632, Cooner Wire) was implanted into the selected muscles. In the right hind limb (contralateral to the recording site in the motor cortex), the electrodes were implanted in m. tibialis anterior (TA), the lateral head of m. gastrocnemius (GL), m. soleus (SOL), and in one cat, m. vastus lateralis (VL). In addition, the electrodes were implanted into the left and right m. triceps (TRI) and the lateral head of left m. gastrocnemius. The electrode placements were verified by stimulation through the implanted wires before closure of the incision. The EMG wires were led subcutaneously and connected to sockets on the head base. The copper wire connected to the skull screws served as a common ground.

**Cell identification and recording**

After several days of recovery, experiments were initiated by placing the animal in a head-restraining device and restraining its head by the base that was attached to the skull during surgery. First, cats were encouraged to take a “sphinx” posture (Fig. 1). After the cat rested in this posture for several minutes, the base was fastened to an external frame so that the resting position of the head was approximated. After several training sessions of increasing duration, the cats were quietly sitting in the “sphinx” position during recording sessions. They did not seem to be disturbed by restraint because they frequently fell asleep.

Neuronal activity was recorded extracellularly using either platinum-tungsten quartz-insulated microelectrodes (40 µm OD) pulled to a fine tip and mechanically sharpened (using a diamond grinding wheel, Reitboeck 1983) or commercially available tungsten varnish-insulated electrodes (Frederick Haer and Co.). The impedance of both types of electrodes was 1–3 MΩ at 1,000 Hz. An electrode was advanced into the cortical tissue through a hole in the plastic plate above the motor cortex. A miniature manual single-axis microinparator, rigidly fixed to the skull, was used to lower the electrode. After amplification and filtering (0.3–10 kHz band-pass) the unitary activity was displayed on the screen of a computer, and also led to an audio monitor. After the electrode reached the depth of the cortex where clear responses of many neurons to hind limb movements could be observed (presumably layer V), two platinum-iridium wires (200 µm OD, insulated with teflon to within 0.4 mm of the tip) were slowly lowered into the medullar pyramid through the guide tubes that were implanted above it. Pulses of graded intensity (in the range of 0.1–1.0 mA, 0.2-ms duration) were delivered through this bipolar electrode. The wires were fixed at the position that was most effective in eliciting antidromic responses in neurons of the motor cortex and served as the pyramidal tract-stimulating electrode during subsequent experiments. The criterion for identification of antidromic responses was the test for collision of spontaneous and evoked spikes (Bishop et al. 1962; Fuller and Schlag 1976; Swallow 1998). All neurons were checked for antidromic activation using this test both before and after recording during scratching. For the purpose of conduction velocity calculation, the distance between the electrodes in the pyramidal tract and the neurons in layer V of the postcruciate cortex was estimated at 51 mm, which included the curvature of the internal capsule and also accounted for the spread of the current and the refractory period at the site of stimulation.

Signals from the microelectrode, EMG electrodes, and acceleration sensor (see Eliciting and monitoring the scratch responses) were first preamplified using miniature custom-made preamplifiers and led to the CyberAmp 380 (Axon Instruments) that served as the main amplifier. Signals were digitized with a sampling frequency of 30 kHz (neurons), 3 kHz (EMGs), and 400 Hz (sensor), displayed on a screen, and recorded to the hard disk of a computer by means of data acquisition hard- and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). The digitized EMG signals were full-wave rectified and smoothed by filters with a time constant of 10 ms.

**Identification of hind limb motor region**

In the cat, the area immediately adjacent to and inside the medial and lateral portions of the cruciate sulcus is considered to be the motor cortex. This is based on a considerable body of data obtained by means of inactivation, stimulation, and recording techniques (Armstrong and Drew 1984b, 1985; Beloozerova and Sirota 1993a; Drew 1993; Martin and Ghez 1985, 1993; Nieoullon and Rispal-Padel 1976; Phillips and Porter 1977; Vicario et al. 1983), as well as on histological considerations (Ghosh 1997; Hassler and Muhs-Clement 1964; Myasnikov et al. 1994). The fine mapping of the body parts in the cortex varies in different subjects, however (Hassler and Muhs-Clement 1964; Myasnikov et al. 1997). To identify the hind limb representations of the motor cortex in each subject, three approaches have been used: somatic receptive field mapping, observation of neuronal activity during voluntary movements, and intracortical microstimulation.

The area just caudal to the medial part of the cruciate sulcus, where 1) the neurons responded to somatic stimulation of the hind limb, 2) the neurons were activated during spontaneous movements of the hind limb, and 3) microstimulation (trains of ten 50-µA cathodal pulses of 0.2-ms duration at 200 Hz) resulted in movements of the hind limb, was considered to be the hind limb motor area. In addition, postmortem positions of recording tracks in the motor cortex were estimated in relation to the reference marks; they were found to be grouped in the area 4γ (Fig. 2).

**Receptive field classification**

The somatic receptive fields of the neurons were examined in the animals resting with their head restrained. Stimulation was produced...
by palpation of muscle bellies, tendons, etc. and by passive movements of joints. The size of receptive fields was determined by listening to the audio monitor and measuring the entire area from which action potentials could be elicited. A directional (flexion-extension) selectivity was assessed by comparing the number of spikes elicited by stimulation in the optimal direction and the opposite direction.

Eliciting and monitoring the scratch responses

In the freely behaving cat, the posture assumed during scratching varies substantially. Cats can scratch different parts of the upper body and the head, and they can do all of this while lying, sitting, or standing. The scratch responses also include head movements toward the active hind limb. To have standard scratch responses, we accustomed the cats to maintain a sitting “sphinx” posture while the head was immobilized. The scratching was elicited by pressing on or by slight rubbing of the external surface of the right ear near the head implant with a cotton swab, while the head was restrained.

The scratch responses were monitored using Visualeyez system (3D Real Time Motion Capture and Analysis System, Phoenix Technologies). It detects the positions in three dimensional (3D) space of light-emitting photodiodes and makes calculations of various kinematic parameters. Wide-angle (140°) light-emitting photodiodes (5 mm diam) were attached to the different sites of the right hind limb (Fig. 1). 4 ventral iliac spine, 2 greater trochanter, 3 approximate knee joint center, 4 lateral malleolus, 5 base of the fifth metatarsal, and 6 end of the distal phalanx of the middle digit, as well as to the skin in a couple of places) to a commutation unit. The frequency of frame sampling was 120 Hz. Joint angles were encoded the sequential number of video frames and appeared on one

by a vertical line and pointed to by an arrow. Square approximately indicates area shown in the photomicrograph in B, COR, coronal sulcus; PRS, presylvian sulcus, B: photomicrograph of a frontal section through the motor cortex, stained with cresyl violet. Layers of cortex are separated by dashed lines and numbered; solid line indicates gray matter-white matter border. Two of the clusters of giant cells in layer V that are characteristic for area 4 are circled. Arrow shows lesion made by the electrode.

FIG. 2. A representative example of a reference electrode track made in the caudal bank of the left cruciate sulcus (CRU) in the motor cortical area 4g, the hind limb representation. A: drawing of a frontal section through the caudal bank of the cruciate sulcus. Reference track made with a thick electrode is shown by a vertical line and pointed to by an arrow. Square approximately indicates area shown in the photomicrograph in B, COR, coronal sulcus; PRS, presylvian sulcus, B: photomicrograph of a frontal section through the motor cortex, stained with cresyl violet. Layers of cortex are separated by dashed lines and numbered; solid line indicates gray matter-white matter border. Two of the clusters of giant cells in layer V that are characteristic for area 4 are circled. Arrow shows lesion made by the electrode.

Neuronal data processing

For the protraction stage of the scratch response, the mean activity of individual neurons and of the population of neurons was compared with those at rest and during the rhythmic stage.

For the rhythmic stage, the peak of the accelerometer signal (that lagged the maximum activity in TA by 18 ± 4 (SD) ms or 10 ± 2% of the mean scratch cycle; see RESULTS) was taken as the cycle onset. The duration of each cycle was divided into 10 equal bins, and a phase histogram of spike activity of the neuron in the cycle was generated and averaged over all successive cycles. The Rayleigh test for directionality was used to determine whether the activity of a neuron was modulated in relation to the scratch cycle (Batshelet 1981; Fisher 1993). The histograms were also assessed visually to determine if there was any reason for a modulated cell to fail the Rayleigh test like multiple activity peaks or a sharp trough on an otherwise level activity (see Drew and Doucet 1991). The discharge frequency in a bin was derived according to the method of Udo et al. (1982) that weights the data according to the average of the instantaneous frequency of intervals that fall within the bin, as well as those that overlap with its beginning and end. The phase histograms were smoothed using a moving filter with a span of three. The portion of the cycle where the activity level in the phase histogram exceeded 25% of the difference between the maximal and minimal frequencies was defined as a "period of elevated firing" (PEF) and the remaining portion, as an "inter-PEF" interval (as shown in Fig. 7D). For the PEF and inter-PEF periods, average frequencies were calculated. A degree of periodic changes in a neuron activity was characterized by a coefficient of frequency modulation: M = Fmax − Finter/Fmax, where Fmax and Finter are average frequencies during the PEF and inter-PEF periods, respectively. In addition, the “depth” of modulation was calculated as dM = (Nmax − Nmin)/N × 100%, where Nmax and Nmin are the number of spikes in the maximal and the minimal phase histogram bin, and N is the total number of spikes in the phase histogram. The two measures for the modulation of neuronal activity, M and dM, were needed to enable comparisons with the motor cortex activity during postural corrections, previously characterized by M (Beloozerova et al. 2005), and during locomotion, previously characterized by dM (Beloozerova and Sirota 1993a; Sirota et al. 2005).

The preferred phase of the discharge of each neuron in the scratch cycle was assessed using circular statistics (Batshelet 1981; Fisher 1993; see also Beloozerova et al. 2003a). The occurrence of each spike was presented as a vector of a unit length. The angle (the phase) of this vector was calculated by multiplying the relative position of the spike in the cycle (in portions of the cycle) by 2π. The preferred phase was calculated as the phase of the mean vector, R, divided by 2π. Preferred phases of all individual neurons were plotted against the phase of the scratch cycle to show their phase distribution. The circular SD S of the preferred phase was calculated as: S = 1/2π√−2log(R), where R is the mean vector.

We did not analyze neuronal activity during hind limb retraction to the starting posture because of a very significant variability of movements during this period.

Parametric tests were used when possible for comparisons between groups. When data were categorical, nonparametric χ² or Mann-Whitney U tests were used. The one-way ANOVA test was applied to characterize effects of categorical factors on a variable. Unless indicated otherwise, for all mean values, the SD is given. For all the tests, the significance level was set at P = 0.05.
Histological procedures

At the termination of the experiment, cats were deeply anesthetized with pentobarbital sodium. Several reference lesions were made in the region of motor cortex from which neurons were sampled. Positions of EMG electrodes in the muscles were verified. Cats were perfused with isotonic saline followed by a 10% formalin solution. Frozen brain sections of 50 μm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. The position of stimulation electrodes in the medullary pyramids was verified by observation of electrode track gliosis. Positions of recording tracks in the motor cortex were estimated in relation to the reference lesions.

RESULTS

Neuronal database

Data on neuronal activity were collected from a total of 26 tracks through the hind limb representation in the left motor cortex: 10 tracks in cat 1, 8 in cat 2, and 8 in cat 3. A representative section through the area of recording is shown in Fig. 2. Figure 2A shows the position of a reference track (made with a thick electrode) on a drawing of the coronal section through the caudal bank of the cruciate gyrus. The track proceeds vertically and ends in the caudal fold of the cruciate sulcus, ~4 mm below the cortical surface. Figure 2B shows a photomicrograph of this track where one can see clusters of giant cell bodies (circled) in the layer V of the cortex that is characteristic for the area 4γ. The track ends right beneath the layer of these giant cells. The activity of the neurons was recorded in the layer V. This was determined by identification of the PTNs known to populate only this layer. The noIDs were collected not more than 200 μm away from the identified PTNs.

The activity of a total of 110 neurons was recorded, with 65 of the cells identified as PTNs. The distribution of axonal conduction velocities of PTNs was bimodal, with approximately two-thirds of the neurons having "fast" conduction velocities in the range of 40–70 m/s (latent periods of 1.3–0.8 ms), and one-third having "slow" conduction velocities, in the range of 8–40 m/s (latent periods of 6.3–1.3 ms). At rest, all the neurons were active, and the average discharge rate was 11.7 ± 7.8 imp/s (range, 0.4–40 imp/s). The fast-conducting PTNs were on average more active than the slow-conducting ones [15.6 ± 1.4 vs. 9.9 ± 0.9 (SE) imp/s; P < 0.05, t-test].

Protraction stage

In the cat sitting with the head restrained, the protraction stage of the scratch response began with a delay of 0.5 ± 0.3 s after an application of the stimulus (here and below, kinematics data are averaged across all trials and all subjects). It included lifting the hind limb on the side of stimulation and its protraction toward the stimulated site. In addition, cats bent the trunk so as to displace the pelvis laterally to the side of stimulation and extended the ipsilateral forelimb. This pattern differed from the normal one in that the head, because of being restrained, could not move toward the protracted hind limb. At the maximal protraction, the limb did not reach the stimulated area but only the upper back/shoulder area, which the limb then rhythmically scratched (Fig. 1).
protraction and the rhythmic stages, usually only the protraction stage was present, often with only a minute hind limb protraction, and cats attempted to remove the stimulus using the forelimbs.

In the sequence of rhythmic scratching movements, the number of cycles varied from 13 to 150 and was on average 28 ± 16. The cycle duration ranged from 130 to 240 ms and was on average 180 ± 30 ms. Figure 5A shows a 3D stick diagram of one representative scratch cycle. The peak-to-peak movement was 9 ± 1 mm at the pelvis, 11 ± 1 mm in the hip, 22 ± 3 mm in the knee, and 34 ± 5 mm in the ankle, whereas toe movement was 70 ± 10 mm. Figure 5B shows joint angles during three scratch cycles. These rhythmic scratching movements were accomplished by the following angular excursions in the hind limb joints: 3 ± 1° (peak-to-peak) in the hip joint, 11 ± 2° in the knee joint, 32 ± 3° in the ankle joint, and 21 ± 6° in the MTP joint. Thus during rhythmic scratching, most of the motion occurred in the ankle and MTP joints, with much less in the knee joint, whereas the hip joint was fairly stable. In Fig. 5B, one can see that both ankle and knee joints flexion movements were briefer than extensions and that the maximal flexion in the knee joint occurred earlier than that in the ankle joint. The maximum of ankle acceleration occurred at the beginning of ankle flexion. The MTP joint had two periods of plantar flexion during a cycle: one right before the ankle extension and another one during ankle flexion. Similar results were obtained in all trials with the flexion movement in the ankle and knee joints comprising, respectively, 35 ± 3 and 39 ± 4% of the cycle, and the maximal flexion in the knee joint occurring 10 ± 3% of the cycle earlier than in the ankle joint.
During rhythmic scratching, muscles of the ankle joint were modulated in the rhythm of scratching. Figure 5 shows the activity of TA and GL muscles during three cycles along with trajectories of joint movements and the accelerometer signal. The activity bursts in TA and GL strictly alternated. After the peak in GL that ends rather abruptly, the TA burst starts sharply with a high-amplitude discharge. Figure 5C shows a 3D presentation of toe tip (Fig. 5A, point 6) trajectory during two of the cycles shown in Fig. 5B. By comparing these fragments, one can see that the abrupt end of the GL burst and the sharp start of the TA burst occurred about 10 ms before the maximal extension in the ankle (Fig. 5B), which is right before the end of the toe tips downward movement (Fig. 5C). In contrast to the abrupt transition from GL to the TA burst, the reverse transition was rather gradual, it included a period of weak activity in both muscles (Fig. 5B). The TA burst gradually declined while the GL increased at about the time of maximal ankle flexion (Fig. 5B), right before the tip of the toes moved down (Fig. 5C). Figure 5D shows the activity in muscles TA (triangles) and GL (circles) presented in a form of averaged normalized phase histograms. Vertical bars: SE.
This pattern of muscle activity was typical for vigorous scratch responses. It could vary considerably, however, depending on the amplitude of activity in the muscles and the corresponding amplitude, acceleration, and frequency of the movements. While the duration of activity bursts in GL, SOL, and TA were similar during vigorous scratching, activity bursts in GL and SOL tended to become shorter, whereas those in TA tended to become longer during less vigorous scratching that sometimes occurred at the beginning or the end of the responses (Fig. 6). Despite a variation in the relative duration of the TA and the GL bursts, the transition from the GL to the TA burst was always sharp and time-locked to the maximum ankle acceleration, preceding it by 18 ± 4 ms or 10 ± 2% of the cycle (Fig. 5B, C). In contrast, timing of the TA to the GL burst transition varied considerably and in the “weak” cycles could occur as late as time-point 19 (or 3) in Fig. 5, B and C; that is, almost at the end of ankle extension and in the middle of the toe downward movement. In a number of cases, scratching cycles with no detectable extensor activity were observed. One can thus suggest that in the weak cycles the paw down movement is largely a passive movement.

During the rhythmic stage, the mean activity of the neurons (PTNs and noIDs combined) was 19.5 ± 20 imp/s, which showed a tendency to be higher than during protraction stage (16.1 ± 22.8 imp/s) but did not reach the level of statistical significance. It was significantly higher, however (P < 0.05, t-test), than the mean activity at rest (11.7 ± 7.8 imp/s). This was caused by an increased activity of 53% of the neurons, whereas the activity of 33% of them decreased. Compared with the protraction stage, the activity of 42% of the neurons (PTNs and noIDs combined) increased and that of 16% decreased (noIDs dominated in the latter group). Among the neurons that were not active during the protraction stage in both PTN and noID subpopulations (n = 27), the great majority were also not very active during the rhythmic stage, either being completely silent (n = 14) or emitting spikes at rates of 0.3–2 imp/s (n = 6), comprising together 18% of the sample. A typical example of a neuron that was active at rest but silent during scratching is shown in Fig. 7A. Before, in between, and after each of the two episodes of scratching, the neuron was active at rates of 30–40 imp/s. This discharge stopped each time at the beginning of the hind limb protraction (shown by an arrow), and restarted only during the last scratch cycle. In general, both PTN and noID neurons tended to be either active (rates > 2 imp/s) or inactive (rates < 2 imp/s) in both the protraction and the rhythmic stages of the response, and the coefficient of correlation between their activity in these two stages was high (0.75; P < 0.05). However, there were 14 (13%) cells (mostly PTNs) that were only active during the rhythmic but not the protraction

FIG. 6. A change in the pattern of muscle activity depending on duration of cycle during 1 scratch response. While in the middle of the response, duration of bursts in TA, GL, and SOL are nearly equal, and TA and GL bursts alternate to take approximately one-half of the cycle each. During slower scratching at beginning and end of response, TA bursts are much longer than those in GL or SOL.

FIG. 7. Representative examples of activity of cortical neurons during the rhythmic stage of scratching. A: in both of the 2 short scratch responses, discharge of PTN 556 stopped at the beginning of the protraction stage (indicated by arrows). Cell remained completely silent throughout this stage and also in the rhythmic stage in both responses; both times, activity recovered only during the last scratch movement. Designations as in Fig. 4. During the rhythmic stage, 24% of neurons were completely silent or discharged at rates ≤2 imp/s. B: activity of PTN 554 was scratch cycle–related: neuron discharged 4–6 spikes in between bursts of activity in muscles rTA and rGL and was silent when the muscles were active. C: discharge of this neuron in 40 cycles of scratching is shown in a raster format. Periods of activity of TA and GL muscles are indicated below graph. D: discharge of the neuron is summarized in a frequency phase histogram; period of elevated activity (PEF; see METHODS) is indicated; interrupted line shows level of activity at rest; vertical scale bar equals 20 imp/s. During the rhythmic stage, discharges of 61% of the neurons were scratch cycle–related.
stage, whereas 4 (4%) cells behaved the other way around. During rhythmical stage, the fast-conducting PTNs were on average more active than the slow-conducting ones (24.9 ± 3.9 vs. 13.1 ± 2.6 imp/s; \( P < 0.05, t\)-test). In total, 76% (84/110) of the neurons (PTNs and noIDs combined) were active during the rhythm stage at rates >2 imp/s, high enough to enable determination of a relationship of their activity to the 180 ± 30 ms cycle of scratching.

During the rhythmical stage, the activity of each neuron was analyzed in ≥20 cycles (70 ± 50 cycles). The activity of 61% (67/110) of the neurons (PTNs and noIDs combined) was modulated in the rhythm of scratching, as determined by the Rayleigh test (see Methods); that is, it was greater in one phase of the cycle and smaller in another phase. This was 80% (67/84) of the total active neuronal population. An example of a modulated neuron is shown in Fig. 7B. The PEF of the neuron started after the peak of TA activity and terminated slightly before the GL peak. The discharge of the neuron in 40 cycles of scratching is shown in Fig. 7C in a raster format. One can see that, although there was cycle-to-cycle variation, the discharge tended to peak in the first half of the cycle. The discharge of this neuron is summarized in a frequency phase histogram in Fig. 7D, and the PEF period (see Methods) is indicated. The percentage of modulated neurons was 52% in the PTN group and 71% in the noID group; this percentage difference was significant (\( P < 0.05, \chi^2 \) test).

The degree of periodic changes in a neuron’s activity was different in different neurons. Across both PTNs and noIDs subpopulations, the depth of modulation, \( dM \), spanned the range from 5 to 25% and on average was 12.1 ± 5.3%. The mean frequency in the PEF was 27.7 ± 17.2 imp/s (PTNs and noIDs combined). The mean frequency in between the PEFs was 18.1 ± 21.7 imp/s (higher than the activity at rest, 11.7 ± 7.8 imp/s, \( P < 0.05, t\)-test). The coefficient of modulation across both PTNs and noIDs, \( M \), was in the range of 20–91%, and on average was 58.2 ± 19.3%.

Different neurons (both PTNs and noIDs) had different preferred phases in the scratch cycle. Figure 8A shows the distribution of the preferred phases (diamonds) for all modulated neurons along with their circular SDs. These are superimposed on the PEFs of activity of the neurons shown as black rectangles. Most neurons (both PTNs and noIDs) had one PEF per cycle, with the PEF ranging from 10 to 80% of the cycle and most values in the 50–70% range. Seven cells had two PEFs and one cell had three PEFs in the cycle. One can see that the preferred phases and the PEFs of the neurons are distributed nearly evenly over the cycle. The constancy of the population activity over the cycle is also reflected in the phase histogram of the number of active neurons (Fig. 8B) and in the mean frequency phase histogram (Fig. 8C). The slight changes over the cycle that are seen in these phase histograms are not statistically significant, and at each phase, ~50% of the motor cortical population was active with an average frequency of 20–25 imp/s.

The neurons (both PTNs and noIDs) with preferred phases during the TA burst in the rhythm stage (pref-TA) were on average more active during the protraction stage compared with the neurons with preferred phases during the GL burst (pref-GL) in the rhythm stage. During the protraction stage, the mean frequency in the pref-TA group was 23.0 ± 4.8 imp/s, and it was 10.2 ± 3.0 imp/s in the pref-GL group (\( P < 0.05, t\)-test).

**Responses of neurons to somatosensory stimulation**

The somatosensory receptive fields were tested in a total of 64 cells. Of these, 90% of the neurons had a receptive field, and fields of 83% of them were excitatory. Receptive fields were confined to toes in 14 cells (22%), to paw in 13 cells (20%), to
ankle in 14 cells (22%), to knee or hip in 6 cells (9%), and 11 cells (17%, including 5 inhibited cells) reacted to whole hind limb manipulation. In 21 responding neurons, responses had a directional preference: 12 neurons (19%) preferred flexion to extension, 9 neurons (14%) preferred extension to flexion. All of the fields were “deep”: the cells responded to palpatation of muscles or movements of joints, or both, but not to stimulation of fur alone.

During the protraction stage of the scratch response, cells that at rest were activated by ankle or knee extension or by hip or MTP flexion, the movements comprising hind limb protraction (Fig. 3B), could be either activated (n = 3) or inhibited (n = 5). At the same time, cells that at rest responded to the opposite movements (ankle or knee flexion, or hip or MTP extension) could be also either activated (n = 4) or inhibited (n = 4). Among six cells with no somatosensory receptive fields, two were activated during the protraction stage, two were inhibited, and the activity of two did not change. Although the activity of four of the five cells that were inhibited by somatosensory stimulation at rest decreased during the protraction stage, the activity of one cell increased.

We also did not find any simple correlation between neuronal responses to somatosensory stimulation in resting position and their activity during rhythmic scratching. Of nine neurons responding to passive flexion in ankle, hip, or the whole limb, two neurons had their preferred phases in the first half of the scratch cycle (i.e., during knee and ankle flexion; Fig. 5B), three neurons had their preferred phase in the second half (i.e., during knee and ankle extension), and the activity of four neurons was not scratch-related (2 of these cells were silent during scratching). Similarly, of eight neurons responding to passive extension of ankle, hip, or the whole limb, three neurons had the preferred phase in the first half of the scratch cycle (i.e., during knee and ankle flexion), two neurons had the preferred phase in the second half (i.e., during knee and ankle extension), and the activity of three neurons was not scratch-related (2 of these cells were silent during scratching). Finally, no peripheral receptive fields were found in six neurons; nevertheless, three of these neurons were strongly modulated during scratching. Also, of five neurons inhibited by passive somatosensory stimulation, the activity of four was well modulated. In addition, we used a one-way ANOVA test to determine if there was a dependence of the parameters of neuron activity during the scratch response on their receptive field properties. The receptive fields were grouped in five categories: toes, paw, ankle, knee, and hip. The discharge frequency at rest and during scratching, the depth of frequency modulation, and the preferred phase were tested. These comparisons revealed no dependence of any of the parameters on the neuronal receptive fields; in all cases, the probability of the null hypothesis was >0.3.

DISCUSSION

Scratch response

We found that, in many aspects, the scratch response with the head restrained that we examined was very similar to natural, unrestrained scratching of cats. Table 1 compares kinematics and muscle activity data obtained in both unrestrained cats (Kuhta and Smith 1990) and head-restrained cats (this study). From Table 1, one can see that a number of general characteristics of the response such as the number of stages, the frequency and duration of the cycle, and the pattern of activation of ankle joint muscles were very similar in both conditions. Also, several more specific parameters such as the timing of movements in different joints, the relative speed of the movements, and the relative duration of GL burst also were very close.

The scratch response in head-restrained conditions did differ from the natural one, however, because of the head fixation. In unrestrained cats, the protraction stage of the response includes bending of the spine, hind limb protraction, and turning of the head toward the limb (Carlson-Kuha and Smith 1994; Kuhta and Smith 1990; Sherrington 1910). Only some components of the protraction stage were present in head-restrained cats, namely, the spine bending and the hind limb protraction. Under head restrained conditions, the hind limb paw did not reach the site of stimulation around the ear, and scratched a closer area—the upper back and shoulder. On the other hand, the head restrained cats were quite able of reaching their ears with the hind limb when moving this limb voluntarily. This observation suggests that it was not pure mechanical reason that prevented the paw from reaching the ear during scratching. It was also not the unwillingness of the cats to scratch the area near the implant because while unrestrained they did scratch it.

The scratching response in the cat presents an example of a motor behavior that consists of several rigid elementary components used in a combination. One can suggest that after a stimulus is applied, calculations of the necessary posture are made separately for the two components: 1) the limb position, and 2) the head position. Considerable errors in component 2) are not compensated by changes in component 1. With even stronger restraints, when not only the head but also the pelvis of a decerebrate cat was fixed in a frame, the hind limb movement did not compensate for the pelvis fixation and the hind limb paw performed scratching movements in the air without touching the skin (Baev 1981; Deliagina et al. 1975, 1981). In our experiments, cats could bend the spine and scratch the body surface. The inability of cats to reach the immobilized head, which mechanically was still within the reach, is similar to the inability of spinal frogs, during wiping, to remove an irritant from a limb if it is fixed away from the normal zone for wiping (Giszter et al. 1989; Kargo and Giszter 2000). At the same time, it was shown that, if an already initiated movement is obstructed in either spinal frogs or intact cats, they all are capable of an on-line correction of the hind limb trajectory (frogs) or force (cats) (Kargo and Giszter 2000; Kuhta and Smith 1990). This suggests that the scratching/wiping “execution mechanism” is more environmentally sensitive than the “planning mechanism.” In spinal frogs, the construction of wiping behavior from a number of motor primitives has been explored in a series of studies (Giszter et al. 1989; Kargo and Giszter 2000; Sergio and Ostry 1993). It has been also shown that scratching behavior in turtles (Stein 2005), locomotion (Orlovsky et al. 1999), balance (Ting and Macpherson 2005), and withdrawal reflex (Levinsson et al. 1999) in cats, as well as a number of other motor behaviors in a variety of animals, are constructed from more or less fixed motor synergies (Bernstein 1967). The inability of cats to develop a compensation for the head restraint over several weeks of daily experiments, so the hind limb could reach the
Table 1. Comparison of scratch responses in unrestrained and head-restrained cats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unrestrained Cats</th>
<th>Head-Restrained Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Stages in the response</td>
<td>Three stages in the response, regardless of posture</td>
<td>Three stages in the response in sitting position</td>
</tr>
<tr>
<td>2 Posture</td>
<td>Standing</td>
<td>Sitting</td>
</tr>
<tr>
<td>3 Hind limb movements during protraction</td>
<td>Hind limb is flexed in the hip, knee, and ankle; knee flexion is then replaced by extension</td>
<td>Hind limb is flexed in the hip, extended in the knee and ankle</td>
</tr>
<tr>
<td>4 Number of cycles</td>
<td>1–60, average 13 ± 9</td>
<td>13–150, average 28 ± 16</td>
</tr>
<tr>
<td>5 Scratching frequency</td>
<td>3.7–8.0 cycles/s</td>
<td>4.2–7.7 cycles/s</td>
</tr>
<tr>
<td>6 Cycle duration</td>
<td>177 ± 31 ms</td>
<td>180 ± 30 ms</td>
</tr>
<tr>
<td>7 Trajectories of the hind limb joints</td>
<td>Circular in MTP and ankle (Fig. 5A)</td>
<td>Circular in MTP and ankle (Fig. 5A)</td>
</tr>
<tr>
<td>8 Hind limb downward movements</td>
<td>Extension in the hip, knee, and ankle, with ankle extension dominating</td>
<td>Extension in the hip, knee, and ankle, with the ankle extension dominating</td>
</tr>
<tr>
<td>9 Hind limb digit movement</td>
<td>Flexed during downward paw movement</td>
<td>Flexing during downward paw movement (Fig. 5, B and C)</td>
</tr>
<tr>
<td>10 Hind limb upward movements</td>
<td>Flexion in the hip, knee, and ankle, with ankle flexion dominating</td>
<td>Flexion in the hip, knee, and ankle, with ankle flexion dominating</td>
</tr>
<tr>
<td>11 Timing of movements in different joints</td>
<td>Knee joint reaches the most flexed or most extended position before ankle, with the hip lagging both</td>
<td>Knee joint reaches the most flexed or most extended position before ankle, with the hip lagging both</td>
</tr>
<tr>
<td>12 Speed of extension and flexion</td>
<td>In knee and ankle, extension is slower than flexion</td>
<td>In knee and ankle, extension is slower than flexion</td>
</tr>
<tr>
<td>13 Posture</td>
<td>Combined lying (21%), sitting (62%), and standing (17%)</td>
<td>Sitting (100%)</td>
</tr>
<tr>
<td>14 Flexor activity during protraction</td>
<td>A single long burst of activity</td>
<td>A single long burst of activity</td>
</tr>
<tr>
<td>15 Extensor activity during protraction</td>
<td>GL and SOL are inactive</td>
<td>GL and SOL are inactive</td>
</tr>
<tr>
<td>16 Flexor and extensor activity patterns</td>
<td>Bursts in TA and GL alternate, SOL starts during TA burst and stops during GL burst</td>
<td>Bursts in TA and GL alternate, SOL starts during TA burst and stops during GL burst</td>
</tr>
<tr>
<td>17 Flexor and extensor activity duration</td>
<td>GL burst occupies 40% of the cycle</td>
<td>GL burst occupies 43% of the cycle</td>
</tr>
<tr>
<td>18 Extensor deletions</td>
<td>Cycles with extensor deletions at the beginning or end of a response</td>
<td>Cycles with extensor deletions at the beginning or end of a response</td>
</tr>
<tr>
<td>19 Muscle activity in relation to kinematics</td>
<td>Kuhta and Smith (1990) Fig 10</td>
<td>Compare to this paper Fig. 5, B and C—similar</td>
</tr>
</tbody>
</table>

Information on unrestrained cats (Kuhta and Smith 1990); head-restrained cats (this study).

ear, suggests that hind limb and head components of the scratch response have rather inflexible relationship even in an intact animal. A detailed evaluation of any possible partial adaptations occurring over this time has proven to be difficult because the amplitude of the hind limb protraction heavily depended on such factors as an overall activity of the cat and the ability of experimenter to find a “scratchy” spot for stimulation.

Our observation of scratching patterns with deletions in extensor activity is in an agreement with similar observations made in intact (Kuhta and Smith 1990) and decerebrate cats (Turkin et al. 2003), as well as in turtles (Stein and Daniels-McQueen 2002, 2004) and frogs (Giszter and Kargo 2000), and is well compatible with the modular hypothesis of scratching automatism organization. This hypothesis proposes a structure for spinal rhythm generators that enables cyclic activity without an antagonist activation (Grillner 1981; Jordan 1991).

The duration of the cycle of the rhythmic scratching stage and its structure, i.e., the relative duration of flexor and extensor phases, in our experiments was similar to that in unrestrained cats (Kuhta and Smith 1990). In the spinal and decerebrated cats, during “air scratching,” the cycle is longer and extension occupies a smaller portion of the cycle (Baev 1981; Deliagina et al. 1975, 1981; Esipenko 1988). Kuhta and Smith (1990) have provided a detailed discussion of the reasons for this difference. They suggested that in intact cats the sensory signals from the paw when it is rubbing against the skin cause a prolongation of extension. We have found, however, that extension not only becomes longer, but it also starts earlier in the cycle, rather than terminate later (because of the contact with the skin). This supports a suggestion that the forebrain influences on spinal mechanisms promote the earlier beginning of the extension phase and thereby make it longer and also accelerate the cycle.

Scratch-related activity of the motor cortex

The main finding of this study is that the activity of many neurons from the hind limb representation of the motor cortex, including the pyramidal tract neurons, strongly correlated with both the protraction and the rhythmic stages of the scratch response. Three hypotheses can be considered regarding the sources of activation and inhibition of cortical neurons during scratching.

First, the motor cortex may contain oscillators that during scratching are coupled to the spinal scratching central pattern generator (CPG). It was previously shown that, in cat decerebrated and immobilized preparations, stimulation of the pyramidal tract affects both the timing and the intensity of scratching movements (Degtyarenko et al. 1992). The duration of the cycle increased more than twofold when stimulation was applied during the activity in the L₅ ventral root (that peaks in the flexion phase), while stimulation in the opposite phase had little effect. At the same time, the activity in the L₅ ventral root...
increased by more than twofold. The activity in the L7 ventral root (that peaks in the extension phase) decreased by about 50% if stimulation was applied before the peak. In this study, we showed that the activity of many neurons in the motor cortex, including PTNs, is strongly scratch phase-dependent. Similarly to the activity of spinal interneurons in cats (Arshavsky et al. 1978c; Berkinblit et al. 1978a; Deliagina and Orlovsky 1980) and turtles (Berkowitz 2001; Berkowitz and Stein 1994), the activity of cortical neurons, both PTNs and nonD cells, typically had one PEF per cycle, with PEFs of different neurons rather evenly distributed over the cycle, suggesting a contribution to activation of both flexors and extensors. Thus if 1) the activity of a structure is a behavior cycle-related and 2) a disturbance of this activity leads to a disturbance in the cycle rhythm, than one can consider this structure to be a part of a pattern generator for this rhythmic behavior.

For locomotion, which has many features in common with scratching, cycle resetting by stimulation of the pyramidal tract or the motor cortex also has been previously shown (Armstrong and Drew 1985; Bretzner and Drew 2005; Orlovsky 1972).

Second, the efference copy signals from the spinal CPG may influence the motor cortex. In the study of the CPG for scratching, it was found that all flexor phase spinal neurons were activated during the protraction stage (Berkinblit et al. 1978a,b). They could be a source of efference copy signals for the motor cortex in this stage. Consistent with this view is our finding that the cortical neurons, which were active in the flexor phase of the cycle, were more active during the protraction stage of the response compared with extensor phase neurons. For the rhythmic stage, efference copy signals from the spinal CPG have been shown to play a leading role in the generation of rhythmic scratch-related activity in the neurons of all descending brain stem–spinal tracts (for review, see Arshavsky et al. 1986). These signals reach the cerebellum through spinocerebellar pathways, where they modulate the activity of its cortex and nuclei. In turn, the cerebellum modulates the activity of neurons of descending tracts: vestibulospinal, reticulospinal, and rubrospinal. It is reasonable to suggest that the motor cortex, and the PTNs in particular, are also driven by efference copy signals from the spinal CPG. Indeed, it has been shown for locomotion that interruption of the cerebello–cortical path (Rispal-Padé and Grangetto 1977) by lesioning the ventrolateral nucleus of the thalamus leads to a significant loss of locomotion-related modulation in the motor cortex (Beloozerova and Sirota 1988, 1998).

Third, afferent signals from the receptive field of the scratch reflex that activate the spinal CPG for scratching can also activate neurons in the motor cortex. During the rhythmic stage, afferent signals from the scratching hind limb can also rhythmically affect the motor cortex. Our findings, however, are not in favor of a pure afferent drive for the motor cortical activity during rhythmic scratching because we have found no simple correlation between the somatosensory receptive fields of cortical neurons at rest and their activity during scratching.

What other roles in control of scratching in addition to the potential participation in the generation of the rhythm the motor cortex may have?

One can suggest that, during the protraction stage, the changes in the PTN activity contribute to activation of flexor muscles and to inhibition of extensors, whereas inhibition of a part of PTNs liberates spinal mechanisms from a non–task-appropriate excitation. During the rhythmic stage, the almost twofold activation of motor cortical population compared with rest conditions may contribute to a general activation of the spinal scratching mechanisms and also help to maintain the steadily protracted posture of the hind limb.

Rhythmic influences from 61% of individual PTNs on the subcortical motor centers and the spinal cord, arriving in a particular phase of the cycle in the rhythmic stage, may contribute to the phase-specific activation and modification of the brain stem and spinal networks. Although the percentage of rhythmic scratch-related neurons found in this study was smaller than the percentage of locomotion-related neurons in the motor cortex reported earlier (80% in Armstrong and Drew 1984a; 89% in Beloozerova and Sirota 1988; and 94% in Beloozerova et al. 2005), the average depth of their scratch-related frequency modulation (12.1 ± 5.3%) was close to that found previously during locomotion (11.5 ± 0.8% in Beloozerova and Sirota 1993a and 10 ± 0.7% in Beloozerova et al. 2005). This suggests that the scratch-related neurons of the motor cortex do participate in rhythmic scratching to a degree similar to that seen during locomotion. The smaller percentage of scratch-related neurons may be explained by a smaller number of joints and muscles involved in the rhythmic process during scratching compared with locomotion.

One may also suggest that, in the head-fixed conditions, the scratch cycle–related activity of the motor cortical may contain cortical correcting signals aimed to bring the hind limb in contact with the ear. Such a function for the cycle-related activity of the motor cortex was suggested earlier for locomotion over an obstructed terrain (Beloozerova and Sirota 1993a; Drew 1993). Indeed, when a step modification is required on encountering an obstacle, the activity of the neurons in the motor cortex changes dramatically. Determining if during scratching the activity of the motor cortex also contains corrective signals will require a comparison of this activity in the head-free and the head-fixed conditions. What is clear, however, is that, unlike during locomotion, during scratching with the head restrained, the activity of the motor cortex was not sufficient for correction of hind limb movements. This might be caused by a less flexible structure of the scratching mechanism compared with the network for locomotion.

In summary, results of this study suggest that, by changing its activity during both stages of the scratch response, the motor cortex participates in the control of each of them but has no ability to correct hind limb movements if the head is restrained.

Acknowledgments

We thank P. Wettenstein for exceptional engineering assistance and Drs. Grigori Orlovsky and Tom Hamm for critical review of the manuscript.

Grants

This work was supported by National Institute of Neurological Disorders and Stroke Grant R01 NS-39340 to I. N. Beloozerova.

References


Degtarenko AM. Changes in the quantitative characteristics of the efferent activity of the scratching generator when the influence of the descending systems is removed. Neurofiziologiya 22: 123–131, 1990.


