

Spatial Segregation of Different Modes of Movement Control in the Whisker Representation of Rat Primary Motor Cortex

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What is mapped on the surface of the primary motor cortex (M1)? The classic somatotopic map holds true on the level of limb representations. However, on the small scale (at within-limb representations), neither somatotopy nor movement dynamics/kinematics seem to be organizational principles. We investigated the hypothesis that integrated into the body representation of M1 there may be separate representation of different modes of motor control, using different subcortical computations but sharing the same motor periphery. Using awake rats and long intracortical stimulation trains in M1 whisker representation (wM1) revealed that natural-like, rhythmic whisking (normally used for tactile exploration) can be evoked from a posteromedial subregion of wM1. Nonrhythmic whisker retraction, on the other hand, was evoked in an adjacent but more anterolaterally located region within wM1. Evoked whisker retraction was always accompanied by complex movements of the face, suggesting that the respective subregion is able to interact with other representations in specific behavioral contexts. Such associations were absent for evoked rhythmic whisking. The respective subregion rather seemed to activate a downstream central pattern generator, the oscillation frequency of which was dependent on the average evoked cortical activity. Nevertheless, joint stimulation of the two neighboring subregions demonstrated their potency to interact in a functionally useful way. Therefore, we suggest that the cause of cortical separation is the specific drive of subcortical structures needed to generate different types of movements rather than different behavioral contexts in which the movements are performed.

Key words: motor cortex; somatotopy; brain map; central pattern generator; vibrissas; whisking; intracortical microstimulation

Introduction

The surface of the primary motor cortex (M1) holds a somatotopic body map, albeit only on the level of body parts such as the head, limbs, trunk, etc. [human (Penfield and Rasmussen, 1950); monkey (Woolsey et al., 1952); rat (Hall and Lindholm, 1974)]. Within representations of body parts, however, the M1 map appears to be organized in mostly distributed and overlapping patches (for review, see Schieber, 2001). In addition, M1 activity codes for movement parameters such as dynamics and/or kinematics (Georgopoulos et al., 1982; Scott et al., 2001), and neurons can be classified according to the type of somatosensory feedback they receive (Lemon, 1981; Strick and Preston, 1982a,b; Picard and Smith, 1992). However, little evidence has been obtained that these parameters are mapped on the small scale (e.g., in columnar order) (but see Strick and Preston, 1982a,b; Amirikian and Georgopoulos, 2003). Graziano et al. (2002) suggested that the intermingled distribution of parameters obeys the rules of a huge map that encompasses premotor areas and M1 and codes for juxtasonic space. They have shown that this map contains representations of higher order, such as semiautomatic movements associated with specific behavioral contexts, in a nested way (Cooke and Graziano, 2004).

We posed the question whether modes of control, as defined by distinct computations committed to subcortical circuits and association to specific behavioral contexts, may be mapped spatially also within the confines of M1 proper. To this end, we were interested in movements that are under control of M1, and include the same muscles, but can be differentiated by the use of central pattern generators (CPGs) to generate uniform movements. Our question then was whether the representations of such modes of control are spatially separated and, if so, whether their location is integrated into the somatic map of M1 (i.e., whether they are located at neighboring sites). As a model system, we studied the representation of whisker movements in M1 of rats (wM1) (Hall and Lindholm, 1974). Rats perform rhythmic whisking for tactile exploration of their environment as well as whisker movements at arbitrary trajectories. Semiautomatic whisking consists in rhythmic movements at 5–11 Hz generated by a presumptive CPG residing downstream from wM1 (Welker, 1964; Lovick, 1972; Semba and Komisaruk, 1984; Gao et al., 2001; Hattox et al., 2003). Nonrhythmic movements are used to bring the whiskers into arbitrary positions with respect to the face to contact objects (Bermejo and Zeigler, 2000).

Guided by previous results in anesthetized animals that suggested that there is a subdivision of protraction and retraction movements in rat wM1 (Sanderson et al., 1984), we decided to explore whether these two regions may correspond to spatially segregated regions controlling rhythmic whisking presumptively involving a subcortical CPG and nonrhythmic movements. Indeed, using long intracortical stimulation trains in awake animals, we found the hypothesized segregation demonstrating that

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representations of modes of control may be integrated into the somatotopic map of M1.

Materials and Methods

Surgery. Fifteen female Long–Evans and Sprague Dawley rats (body weight, 200–300 g) were used in the present study. All experimental and surgical procedures were performed in accordance with the policies on the use of animals in neuroscience research of the Society for Neuroscience and German Law. Anesthesia was initialized with isoflurane (1–3%). At least 25 min before starting microstimulation, anesthesia was switched to continuous intravenous injection of ketamine S (~2 mg · kg⁻¹ · min⁻¹). The flow of ketamine was finely adjusted such that responses to tail pinches were suppressed, but the anesthesia was light enough to allow for a whisker tremor of very small amplitude typical for ketamine anesthesia of superficial to medium depth (Kisley and Gerstein, 1999; Brecht et al., 2004). This stage of anesthesia was found to be optimal to elicit movements from M1. The animal's rectal temperature was controlled automatically by a feedback circuit composed of a rectal probe and a heating pad and set to 35°C (Fine Science Tools, Heidelberg, Germany). For stimulation of the neocortex, animals were placed in a stereotaxic apparatus, and craniotomy was performed to gain access to wM1 according to coordinates given by Sanderson et al. (1984). For acute experiments, the dura mater was removed, and a single electrode was lowered into the neocortex at a 90° angle with respect to its surface to a depth of 1300 μm using a hydraulic micropositioner (Kopf 650; David Kopf Instruments, Tujunga, CA).

The dura mater was kept intact for animals used in the chronic preparation (four animals). These animals were implanted with a 4 × 4 microelectrode array at a depth of 1300 μm. This target depth was assured regardless of dimpling of the cortex using an optical marker located on the shaft of one of the electrodes. A dimpling of the cortex of up to 300–500 μm during penetration of the array was accepted and was compensated immediately once the target depth was reached by retraction of the electrode array. Dimpling of higher amplitudes was avoided in all cases by reducing the speed with which the electrodes penetrated the brain. A titanium screw placed in the skull between lambda and bregma over the contralateral hemisphere served as the second pole for microstimulation. Up to seven titanium screws (Titan MIKRO-Schraube; Medicon, Tuttlingen, Germany) were placed in the skull to ensure head cap stability. The electrode array was permanently cemented with light curing dental cement (Flowline; Heraeus Kulzer, Hanau, Germany) to the skull screws and to one larger screw extruding vertically from the head cap used later for head posting.

Electrodes. Multielectrode arrays were custom made in our laboratory. Sixteen pulled and ground glass-coated platinum tungsten electrodes (80 μm shank diameter; 23 μm diameter of the metal core; free tip length, ~8 μm; impedance, >1 MΩ; Thomas Recording, Giessen, Germany) were mounted inside a 4 × 4 array of polyimide tubing, setting the tip distance to ~350 μm (HV Technologies, Trenton, GA). The free ends of the electrodes were soldered to Teflon-insulated silver wires (Science Products, Hofheim, Germany), which in turn were connected to a microplug (Bürklin, München, Germany).

Intracortical microstimulation. For extracellular stimulation, bipolar current pulses (duration, 200 μs; cathodal first) were delivered using a programmable stimulator (STG 1008; MultiChannel Systems, Reutlingen, Germany). Short stimuli were composed of a burst of pulses consisting of five pulses at intervals of 2.2 ms. Long continuous pulse trains (350, 700, and 1400 ms) were delivered at frequencies of 60 and 100 Hz in experiments under anesthesia (20–90 μA) and at 60 Hz in awake animals. The choice of frequency was guided by findings in preliminary experiments varying stimulus frequencies of 8, 16, 32, 60, 100, 200, and 500 Hz: the largest amplitudes and duration of movements were typically evoked at ~60 Hz (see Fig. 4D). The amplitude of the stimulus current used in awake animals ranged from 12 to 75 μA.

Data collection. For measurements in awake subjects, rats were handled 3–4 weeks before head cap surgery to adjust them to the experimenter's hand and the experimental set up. Earliest 1 week after implantation accommodation to head fixation was commenced. For this purpose, the rat entered a black tube. The screw mounted on the head cap

could then be grasped gently using tweezers, and the rat's head was fixed without touching its hair or skin. In the head-fixed situation, the head of the rat was located outside the tube while its body remained inside. For intracortical stimulation, the rats were transferred to a dark and acoustically dampened experimental set up. Whisker movements were tracked in a rostrocaudal direction by a linear CCD array illuminated by a two-dimensional infrared laser at a resolution of 11 μm in space and 1.4 ms in time (Bermejo et al., 1998). One of the whiskers was marked with a thin stripe of lightweight gelfoam to facilitate detection. This whisker crossed the scanning line of the sensor at a distance of 13.5–16.5 mm to the rat's face. Because of this inaccuracy, which was not critical for the main conclusions of the present study, we present here whisker trajectory in millimeters on the CCD array (the angular displacement was ~3.6° per millimeter). The range of natural whisking was almost completely contained in the range covered by the CCD array. Some electrical stimuli, however, invariably evoked large amplitude whisker retraction. In these cases, the whisker would escape the range of measurement and result in clipped whisker trajectories (for example, see Fig. 2B). To assess the position and movements of whiskers under these circumstances, video clips at 125 frames/s (MotionScope PCI; Redlake, San Diego, CA) were taken. Qualitative evaluation revealed that retraction amplitudes leaving the sensor's range led to a smooth and very close alignment of the vibrissa to the rat's face. After cessation of the pulse train, the whisker came straight back to the sensor's range, where it was captured again. Videos at the same frame rate were also performed to monitor movements of the face that accompanied whisker retractions. Calculating power spectra from whisking trajectories was done using custom-written scripts in Matlab (MathWorks, Natick, MA).

Results

Vibrissa movement evoked during intracortical microstimulation in the anesthetized rat

Quantitative data about whisking trajectories were assessed from 15 animals under anesthesia (Fig. 1A,B). Four of them were used for detailed spatial mapping using many penetrations covering the whole wM1 (Fig. 1C,D). In seven animals, the focus was on quantitative assessment of whisker trajectories, and another four animals were mapped coarsely during surgery for chronic implantation. Sanderson et al. (1984) reported that wM1 is divided into two subregions, a larger anterolateral one coding for retraction and a smaller posteromedial one coding for protraction, a finding that has never been confirmed since the initial report was published (Gioanni and Lamarche, 1985; Neafsey, 1990; Miyashita et al., 1994; Carvell et al., 1996; Franchi, 2000, 2001). Our present experiments using short stimuli (five current pulses at intervals of 2.2 ms) validate the location of the subregions found by Sanderson et al. (1984) and the qualitative findings of their study. Stimulation of the anterolateral subregion [0.5–4.5 mm anteroposterior (A-P), 0.5–2.5 mm mediolateral (M-L) from bregma; tagged by us as the “retraction-face” region (RF) to describe the effects seen in the awake animal; see below] led to a twitch pulling all whiskers backward in all 15 animals studied (Fig. 1A, light gray trace). The amplitudes evoked were big enough to make them readily detectable by eye. Face movements were not observed under anesthesia. On the other hand, stimulation of a contiguous, posteromedial subregion led to whisker protraction (*n* = 15 animals; 0.5–2 mm A-P, 0.5–1.25 mm M-L; we tagged this region the “rhythmic whisking” region (RW) because it gave rise to such movements in awake animals; see below). In some animals, as the one shown in Figure 1B (light gray trace), the movement was barely seen looking at the trajectory recording (arrow at light gray trace). In other animals, the protraction movement was somewhat bigger and could be discerned by visual inspection. In all 15 cases, protraction elicited by short bursts in RW was of substantially smaller amplitude than retrac-

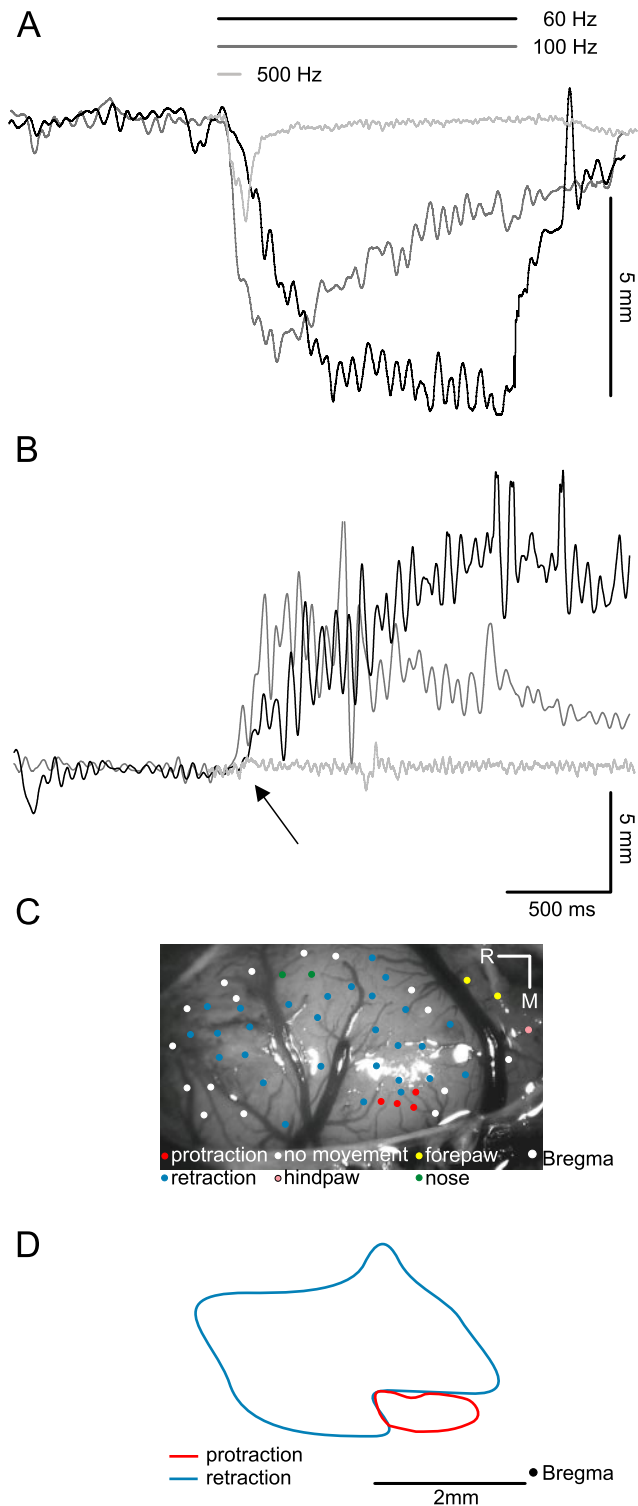


Figure 1. Whisker protraction and retraction are elicited from different subregions within M1 under ketamine anesthesia. *A*, Whisker trajectories evoked from an anterolateral area (RF area). Already, short pulses evoked a visible whisker retraction (light gray trace; 90 μ A). Long stimulation at 100 Hz evoked a maximal retraction, followed by slow relaxation forward toward null position (medium gray trace). Long stimulation at 60 Hz evoked a slower but constant retraction movement (black trace). *B*, Whisker trajectories evoked from a posteromedial area (RW area). Short pulses evoked only a minimal whisker protraction (arrow) sometimes not visible by the eye. Long stimulation at 100 Hz (medium gray trace) evoked a clear protraction that quickly reaches a maximum and then relaxes back toward the null position. Long stimulation at 60 Hz evoked a slower monotonic protraction (black trace). Note that the whisker stayed protracted even after the stimulus command was turned off. *C*, Photomicrograph of the surface of M1 in one animal. Movement of body parts and directions of whisker movements are marked

tion elicited by the same stimulus protocol in RF. In all animals, whisker movements on the contralateral body side to the stimulated hemisphere were evoked. In addition, some stimulation sites (not seen in all animals) evoked bilateral or exclusively ipsilateral movements.

The picture changed completely when we used long pulse trains (1400 ms) for stimulation in both RF and RW. Evoked protraction movements from RW were now of much larger amplitude and duration, making them easily discernable by eye. Long stimulation pulses were applied with two different frequencies (100 and 60 Hz). In both subregions, stimulation at 100 Hz triggered larger initial whisker velocities but resolved into a partial backward movement after reaching a maximum excursion after \sim 500 ms (Fig. 1, medium gray traces). Stimulation at a frequency of 60 Hz, in contrast, brought the whisker in a far protracted or retracted position and kept it there for the whole duration of the pulse train (Fig. 1, black traces). In all cases ($n = 15$ animals) and in both subregions, larger amplitude excursions could be evoked by 60 Hz stimulation than with 100 Hz stimulation. A peculiar characteristic was found for evoked movement from RW: evoked protraction was kept much longer than the duration of the train of stimuli, most prominently so after 60 Hz stimulation (Fig. 1 *B*). Sometimes protraction would stay for tens of seconds after offset of stimulation and only slowly relax back to the position at stimulus onset. In sharp contrast, retraction evoked from RF always relaxed back to the resting position immediately after stimulus offset (Fig. 1 *A*). Using 60 Hz trains of 1400 ms duration, a detailed map of wM1 was established in four animals (Fig. 1 *C*). The location of RF and RW was congruent with the retraction and protraction areas reported by Sanderson et al. (1984). The compound map shown in Figure 1 *D* represents data from all four animals mapped at the resolution and extent as the one shown in Figure 1 *C*, using a total of 220 penetrations. The border between the subregions was well defined in all cases (Fig. 1 *C*). Only occasionally sites at the border evoked a mixed response (containing a short retraction followed by a longer protraction).

Our results so far supported and extended the data of Sanderson et al. (1984). The clear difference in effects using short and long stimulation provide possible explanations why the generation of protraction from this region may have been overlooked by many previous studies. Short trains, as typically used earlier, elicit protraction to a far less degree than retraction (or are not effective at all). Some studies using short stimulation reported unresponsive sites at the medial margin of the whisker representation (Franchi, 2000, 2001). Possibly, these sites were located within RW, but the short stimulation train used may have been insufficient to evoke visible whisker protraction. Second, RW is much smaller than RF, another reason why protraction movements are more difficult to detect. It should be also noted that it was always crucial to keep the animal at a level of anesthesia, characterized by spontaneous low amplitude whisker tremor at \sim 15 Hz (see Materials and Methods for details). As can be appreciated in Figure 1, it was a common finding that this ketamine-induced whisker tremor was greatly amplified in amplitude during both protraction and retraction movements. Optimal stimulus parameters to

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by colored dots at the stimulation sites (R, rostral; M, medial; bregma is indicated by the white dot in the bottom right corner). *D*, Line drawing showing the limits of the compound map for retraction and protraction as found in four animals mapped at a spatial precision similar to *C* (220 sites in total). Scaling and location of bregma is aligned in *C* and *D*.

search for RF and RW in anesthetized animals using visual inspection of the whiskers were 60 Hz pulse frequency, 70 μA stimulus intensity, and 1400 ms train duration. Using these to locate RW and RF, we chronically implanted 4×4 electrode arrays encompassing RW and RF in four rats. These animals were then used to study electrically evoked movements from the two subregions during wakefulness.

Vibrissa movement evoked during intracortical microstimulation in the awake rat

As in anesthetized animals, long pulse trains (duration, 1400 ms) at 60 Hz delivered to RF in awake animals elicited strong whisker retraction at short latencies (<80 ms in all cases) and small current intensities ($\sim 12 \mu\text{A}$). The excursions of the whisker were larger than those obtained under anesthesia. They exceeded the window of the CCD array already at current amplitudes close to threshold (Fig. 2*B*). An even more obvious, qualitative difference to the results in anesthetized animals was that complex movements encompassing several parts of the face typically accompanied the whisker movements. These additional movements involved eye, eye lid, snout, ear, and forepaw, started at short latency, and were disclosed by current intensities close to threshold (Fig. 2*A*) (supplemental movie, available at www.jneurosci.org as supplemental material). They were observed preferentially contralateral to the stimulated hemisphere. To a lesser degree, ipsilateral movements were evoked as well. Using repetitive stimulation revealed that the movements in the initial phase of the stimulation (<200 ms) were highly stereotyped (Fig. 2*A*). In contrast, movements that occurred later in the trial were more variable, indicating a possible contribution of self-initiated action.

In sharp contrast to retraction movements, whisker movements evoked from RW in awake rats differed fundamentally from the protraction seen under anesthesia. Long stimulation in RW generated rhythmic movements at frequencies between 5 and 10 Hz in conscious animals (Fig. 3*A*). The threshold intensities were higher for evoked movements in RW ($\sim 30 \mu\text{A}$) compared with RF ($\sim 12 \mu\text{A}$). The short latency of well under 80 ms in all cases indicated that they were evoked electrically. Notably, these movements were virtually indiscernible from natural whisking movements. The video sequence depicted in Figure 3*B* demonstrates a whisking cycle evoked by electrical stimulation. The protraction phase is approximately four times longer than the retraction phase, a feature typically seen in self-initiated rhythmic whisking. Figure 3*C* compares the trace of a self-initiated whisking cycle just before the onset of the stimulus with one that was evoked by electrical stimulation at short latency (in the same animal but taken from another trial in which there was no whisker movement before the onset of stimulation): shape and duration of the electrically evoked and naturally occurring whisking cycle were nearly identical. Moreover, again in strong

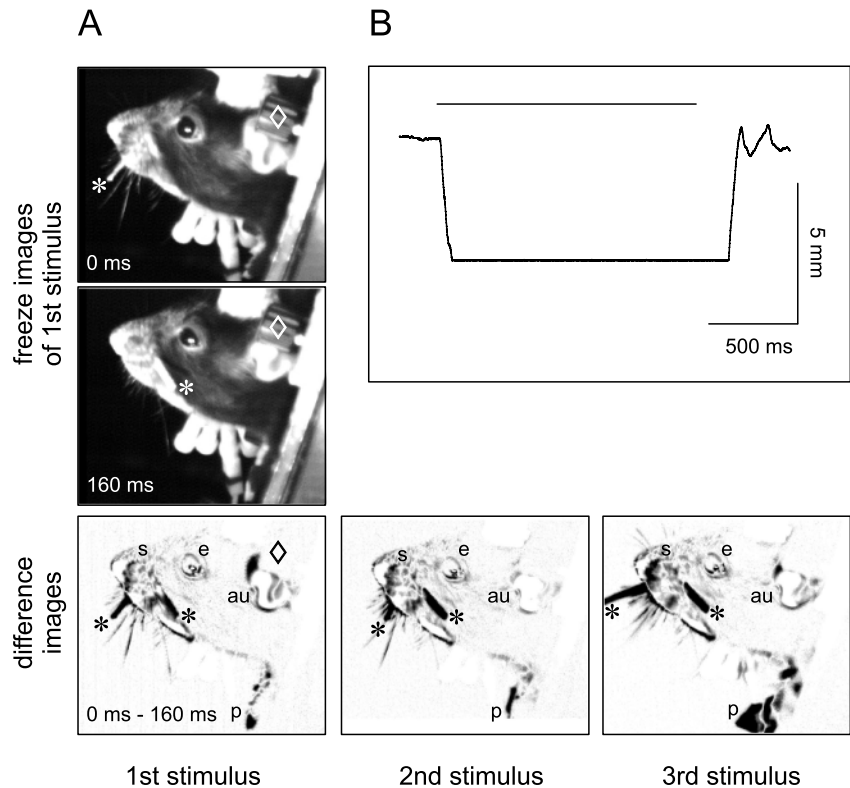


Figure 2. Whisker retraction is elicited from RF in conscious animals. *A*, The top two images show the rat's head at stimulus onset (0 ms) and 160 ms thereafter (60 Hz train at 50 μA). The whiskers have moved backward after 160 ms (asterisks indicate the whisker carrying the gelfoam marker). To make face movements that are well visible in the movie (see supplemental material, available at www.jneurosci.org) evident in the freeze image, we generated an inverted absolute difference of the images at 160 ms from the one obtained at 0 ms (bottom row of images). Black features in the different images are the ones that moved between time 0 and 160 ms. Immobile regions appear white (see, for instance, parts of the restrainer marked by the diamond). It is evident that, together with the whisker (asterisks), snout and whisker pad (s), eye (e), ear (au), and paw (p) moved as well. Three different images from three subsequent stimulation trials are shown to demonstrate the repeatability of the evoked movements (the freeze images shown correspond to the first stimulus). *B*, Typical trajectory of whisker retraction evoked from RF in an awake animal. The whisker was retracted at a latency of ~ 50 ms and quickly left the measuring range of the CCD array. For the entire stimulus duration (horizontal line on top), it stayed pulled up alongside the face. After the end of the stimulus train (indicated by the horizontal line on top), the whisker moved swiftly back to the null position.

contrast to stimulation in RF, rhythmic whisking was never accompanied by face movements even with the highest stimulus intensities used (75 μA) (Fig. 3*B*) (supplemental movie, available at www.jneurosci.org as supplemental material).

These results were obtained at the optimal stimulation frequency of 60 Hz. Higher stimulation frequencies (100 and 200 Hz) led to a strong attenuation of the amplitudes after a few hundreds of milliseconds (Fig. 3*D*). Lower stimulation frequencies led to rhythmic whisker movements, but at higher threshold currents (see Fig. 5*C*, which shows data obtained using a stimulation frequency of 32 Hz; the same site evoked similar rhythmic whisker movements using threshold currents of 30 μA at 60 Hz and 40 μA at 32 Hz; data not shown). Whisking frequency was not consistently modulated by changing current intensity and pulse frequency. The short onset latency of rhythmic whisking indicated that it was evoked by the electrical stimulation rather than initiated voluntarily by the animal. The trajectory of rhythmic whisking later in the train, however, varied slightly in phase from trial to trial. Nevertheless, the evoked rhythmic whisking persisted throughout the stimulation period and terminated closely thereafter, suggesting that also in later phases of stimulation whisking was evoked electrically. This observation was substantiated by variation of the stimulus duration. Figure 4 shows

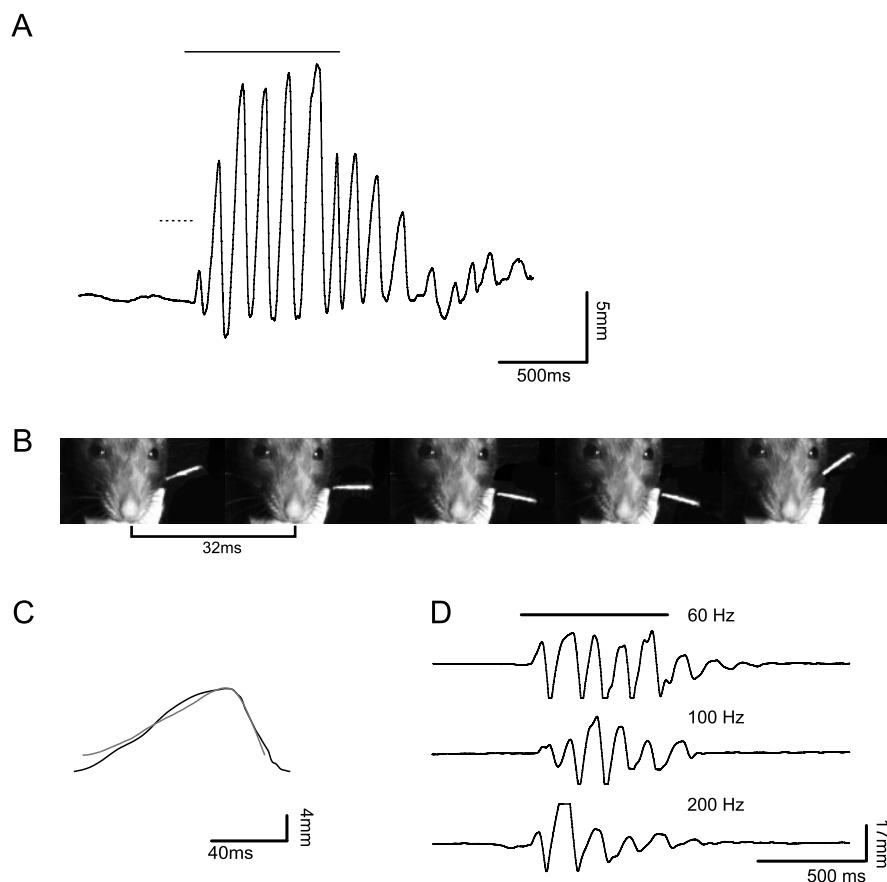


Figure 3. Rhythmic whisking is elicited from RW in conscious animals. *A*, Typical trajectory obtained from long stimulation (60 Hz, 50 μ A) in RW. The average set point in this case was protracted (indicated by the dotted line). *B*, Pictures taken from a video sequence during one cycle of electrically evoked whisking. The movie taken from the front shows the typical asymmetry in duration of protraction (seen in 4 frames) and retraction (last frame). *C*, Traces of one whisking cycle as recorded using the CCD array. The black trace is a self-initiated spontaneous whisk performed by the rat before electrical stimulation started. The gray trace is an electrically evoked one (same rat). Note the similarity in shape and duration. *D*, Comparison of whisker movements evoked from a site in RW at stimulus frequencies of 60, 100, and 200 Hz. Stimulation at 60 Hz evoked rhythmic whisking at constant amplitudes. Higher stimulation frequencies evoked a few whisking cycles at comparable frequency and amplitudes that then (during ongoing stimulation) were followed by cycles tapering in amplitude (most evidently at the highest frequency of 200 Hz).

single trials of evoked whisker movements obtained from electrodes located within RW (Fig. 4*A*) and RF (Fig. 4*B*). In all cases, movements were elicited reliably, and the duration of the movement corresponded to the duration of stimulation (350, 700, and 1400 ms, indicated by the gray fields in Fig. 4). For retraction movements evoked from the electrodes within RF, the correlation of stimulus duration and retraction movement was obvious without further analysis, because the whisker always reentered the range of the CCD array shortly after the end of stimulation (Fig. 4*B*). To obtain a quantitative appraisal of the duration of rhythmic whisking evoked from RW (Fig. 4*A*), we calculated the integral of the whisking trajectory within an interval spanning 0–1400 ms after stimulation onset, after setting the null position of the oscillation to zero, normalizing, and rectifying the trajectory. The integral correlated very well to the duration of movement because the amplitudes and periods of rhythmic whisking were fairly constant within and across trials. The integrals obtained from trajectories during stimuli of short (350 ms; $n = 37$), medium (700 ms; $n = 27$), and long (1400 ms; $n = 27$) duration were statistically different for the three stimulation durations in all three animals tested ($n = 90$; one-way ANOVA, $p < 0.0001$; Tukey's *post hoc* comparison yielded significant differences of integrals evoked by 350 and 700 ms stimulus duration, as well as

between those evoked by stimuli of 700 and 1400 ms duration, $p < 0.01$; one animal was not included in the analysis because trajectories notoriously reached the caudal limit of the detector and were clipped; the qualitative results from this animal, however, were comparable with the three animals analyzed). It should be noted that after RF stimulation whisker movements of variable amplitude, duration, and frequency content (Figs. 4*B*, *D*) as well as face movements (supplemental movie, available at www.jneurosci.org as supplemental material) were observed. We deem it unlikely that rebound activity in RF ensuing after offset of the stimulation is at the basis of these movements, because entirely different types of movements were observed during stimulation (nonrhythmic retraction) and after stimulus offset (rhythmic whisking). Alternatively, prolonged activation of RW after stimulus offset in RF is conceivable. However, the inconsistency (e.g., missing or very short movements after the fourth and eighth trial from the top in Fig. 4*B*) and high variability (lower amplitudes and wider frequency range compared with movements directly evoked from RW) (Fig. 4*D*) render this possibility questionable. Thus, we regard it the most parsimonious explanation that whisker and face movements generated after stimulus offset in RF were self-initiated.

We obtained similar data as described so far from four fully awake, head-restrained animals that had received electrode arrays with tip distances of ~ 350 μ m. The RW and RF regions were observed at sites that corresponded to the mapping under anesthesia. RW is a very small region because rhythmic whisking was generated from only two to three neighboring electrodes in each of the animals investigated. Across the four animals, we obtained rhythmic whisking from 10 sites. The set point of rhythmic whisking was variable and was measured in eight of the sites as the whisker position during a cycle at which the maximum velocity in forward direction was reached (Fig. 3*A*). Averaging across cycles obtained at one site revealed that six sites gave rise to oscillations around a mean set point backward to the null position of the whisker (−4.3, −1.6, −6.3, −7.2, −1.6, and −2.7 mm) and two sites leading to an average protraction of the set point (0.1 and 2.5 mm). The two remaining sites were used for multielectrode stimulation (see below) and were not recorded during single stimulation. Visual inspection of the traces during the experiment indicated slightly protracted set points.

Spatial interactions of electrically evoked movement

The observation that set points of rhythmic whisking vary from site to site suggested that wakefulness may facilitate functional interactions of rhythmic and nonrhythmic movements, perhaps by interaction of RW and RF. To demonstrate that this is a viable possibility, we investigated whether the two types of movements evoked from the different regions can, in principle, be combined

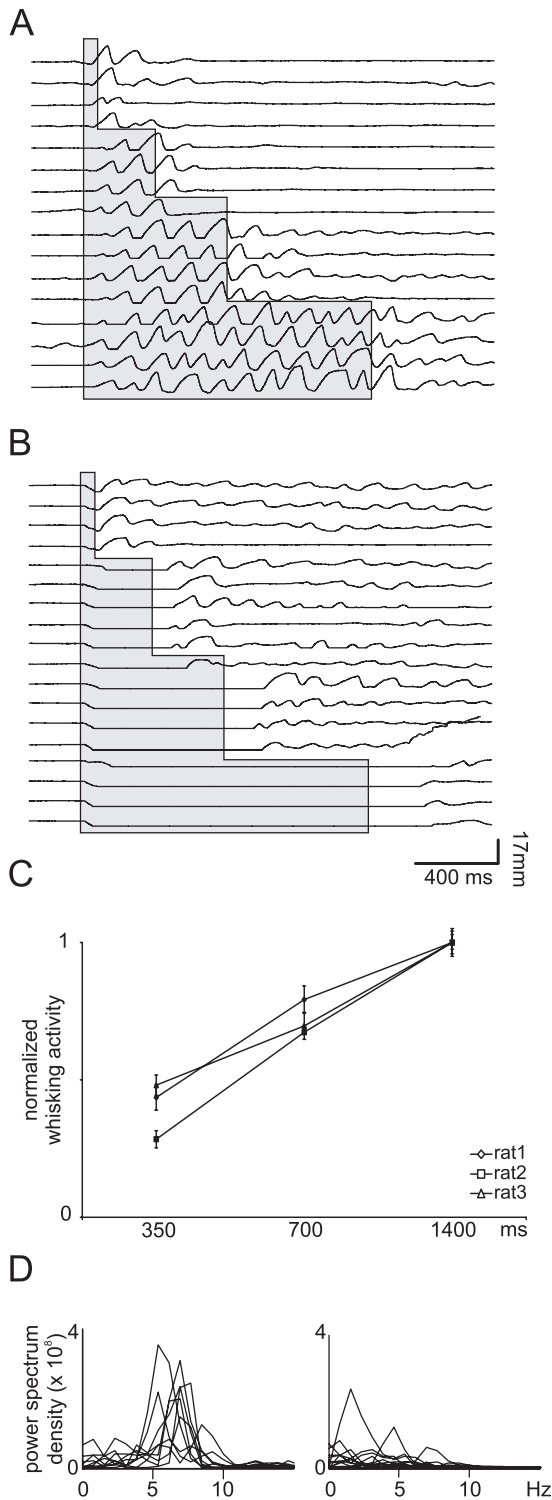


Figure 4. Electrically evoked movements last as long as the stimulus. Stacked single trajectories evoked from a site within RW (*A*) and in RF (*B*) are shown. The gray fields indicate the stimulus duration (350, 700, and 1400 ms; 60 Hz, 50 μ A). Note the highly variable whisking movements after stimulus offset in RF (*D*) (supplemental movie, available at www.jneurosci.org as supplemental material). *C*, Quantitative analysis of duration of rhythmic whisking (as shown in *A*) for three rats. The ordinate plots the integral of a whisking trace from 0 to 1400 ms after stimulus onset after normalizing the trace to its maximum, setting the mean position to zero, and rectifying it. This measure corresponds to the duration of rhythmic whisking and rises monotonically with stimulus duration. *D*, Characteristics of rhythmic whisking are different during stimulation of RW and after stimulation in RF. Left, Overlay plot of single trial power spectra from whisker trajectories during RW stimulation (*A*, gray area). Right, Same type of plot, but data were taken from the period after stimulation in RF (to the right of the gray area in *B*).

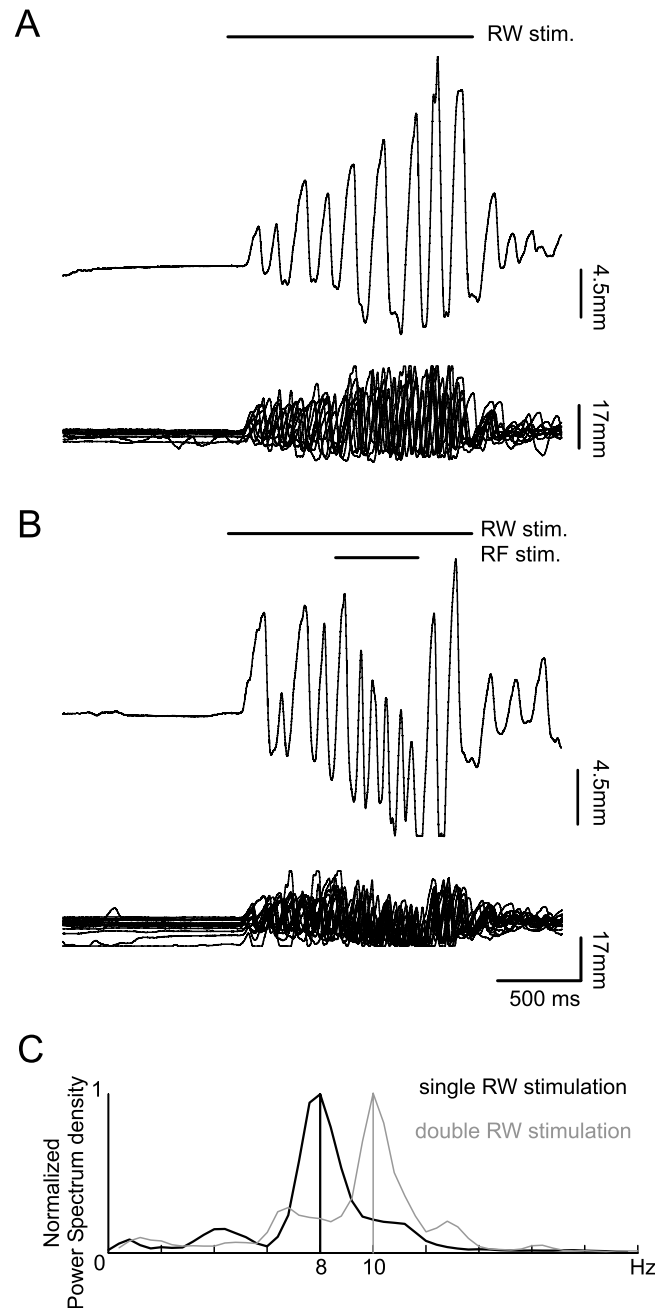


Figure 5. Synchronous activation of RW and RF modulates set point of rhythmic whisking. Synchronous activation of two sites within RW modulates whisking frequency. *A*, Single-electrode stimulation (stim.) from a site within RW (60 Hz, 50 μ A). The bottom plot shows 10 single trials evoked the same way and overlaid. *B*, Double stimulation of the site shown in *A* and a site in RF that gave rise to whisker retraction in a window starting 670 ms after onset of RW stimulation and lasting for 500 ms (60 Hz, 12 μ A). Note the combined effect of rhythmic whisking and retraction after RF stimulation was turned on. The bottom plot shows 13 trials evoked by double stimulation and overlaid. Note that the whisker left the CCD array in several trials, leading to clipping of the trajectory. *C*, Power spectra from single-electrode (black trace) and double-electrode (gray trace) stimulation from sites within RW. Single-electrode stimulation evoked rhythmic whisking at 8 Hz. The rhythm evoked by double-electrode stimulation shows a significant (see Results) elevation of the whisking frequency to 10 Hz.

by synchronous activation. The RW site was stimulated as before to evoke whisking at 5–8 Hz (Fig. 5*A*). The stimulation of the RF site was started at a delay of 670 ms after stimulus onset in RW and lasted for 500 ms (Fig. 5*B*) (individual stimulus pulses occurred at perfect synchrony during the period of double stimula-

tion). Indeed, a combination of effects was observed in the time window of double stimulation (Fig. 5B): rhythmic whisking continued while the additionally evoked retraction shifted the set point of whisking backward in two animals investigated. We conclude from these results that the effects from activation at the RW and RF sites can be combined. Thus, at least in principle, the two subregions may work together to adjust the set point of rhythmic whisking.

Additional evidence that activity from different sites in the cortex can interact to control movements was obtained from synchronous activation of sites within RW that showed whisking close to the resting position in two animals (i.e., trajectories were well contained within the detector's range). Compared with single-electrode stimulation, movements evoked by double-electrode stimulation (at exact synchrony of stimulus commands; 32 Hz, 75 μ A, 1400 ms) left the whisking amplitude unchanged but significantly increased whisking frequency: in the example shown in Figure 5C, the average peak found in the power spectrum of whisker trajectories was 8 Hz ($n = 9$ trials) during single-electrode stimulation and increased to 10 Hz during simultaneous double-electrode stimulation ($n = 12$ trials) (t test; $p < 0.01$). The second animal showed an increment of whisking frequency from 6 to 8 Hz with single-electrode ($n = 18$ trials) and double-electrode ($n = 10$ trials) stimulation, respectively (t test; $p < 0.01$).

Temporal modulation of the stimulus command

We have demonstrated that electrical stimulation in RW can generate rhythmic whisker movements without activating RW cells at whisking frequencies. We next asked how the temporal pattern of stimulation influences whisking rhythm. Figure 6 compares continuous stimulation in RW (60 Hz) (Fig. 6A) with burst stimulation at 5 Hz (Fig. 6B) and 2.5 Hz (Fig. 6C). Each burst consisted of 10 pulses at intervals of 5 ms. The most conspicuous finding was that electrical stimulation invariably evoked whisking at ~ 7 Hz no matter which temporal stimulus pattern was used (as indicated by the power spectrum of the trajectory) (Fig. 6, insets). In fact, virtually the same frequency content was found during continuous and 5 Hz burst stimulation (Fig. 6A,B) ($n = 2$ animals; the cases used showed trajectories well within the detector's range). The lower burst frequency of 2.5 Hz, on the other hand, evoked frequencies ~ 2.5 Hz, but the whisking frequency ~ 7 Hz was contained as well, as reflected by the double-peaked whisking movement evoked by each burst and the two peaks at 2.5 and 7 Hz in the power spectrum (Fig. 6C). Accordingly, the whisking frequency ~ 7 Hz could be isolated by calculating power spectra taken from whisker trajectories in a small window after one individual burst (Fig. 6C, frame in the lower plot). The resulting power spectrum was comparable with the one observed during continuous stimulation. These findings support the idea that the rhythm with which the motor cortex is activated is reflected in its output (Berg and Kleinfeld, 2003a). However, they demonstrate an important additional fact: there is an intrinsic rhythm in the range of natural whisking that is activated always, regardless of the frequency content of the stimulus command.

Discussion

We found that electrically evoked whisker movements in conscious rats fall into two types that could be elicited from separate but adjacent sites within M1: (1) nonrhythmic whisker retraction accompanied with complex face movements; and (2) rhythmic whisking at frequencies typically used by the animals during explorative behavior. In view of the presumptive different engagement of subcortical circuits in the execution of these two types of movement,

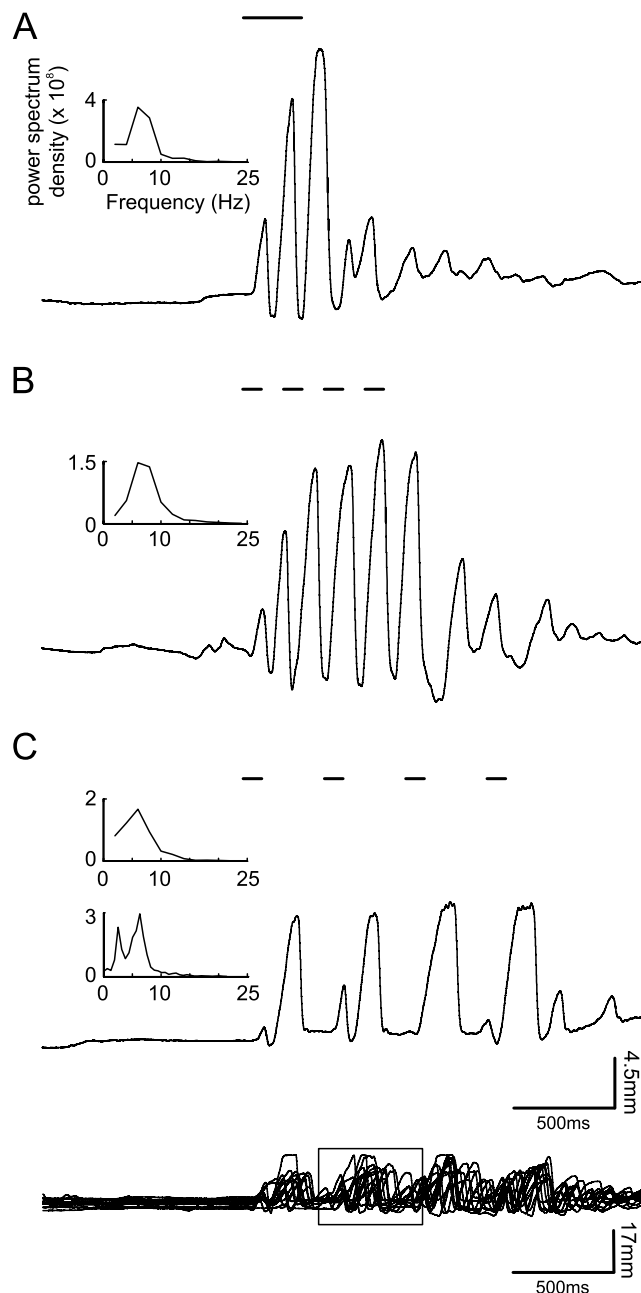


Figure 6. Inherent whisking frequency is evoked regardless of variation in the temporal structure of the stimulus command. The horizontal line on top of the trajectories indicates stimulus duration. The insets depict power spectra of the trajectories. *A*, Continuous stimulation (60 Hz, 50 μ A) leads to rhythmic whisking at ~ 7 Hz. *B*, Burst stimulation (5 Hz) also evokes rhythmic whisking at ~ 7 Hz. *C*, Burst stimulation at 2.5 Hz reflects the stimulus frequency in the whisker movement. However, the rhythmic whisking at ~ 7 Hz is evoked as well (note the double-peaked trajectory evoked by each burst and the double-peaked power spectrum in the bottom inset). Calculating the power spectrum from a narrow time window after one burst (frame in the overlay plot at the bottom, depicting 10 trajectories obtained under these conditions) extracts the whisking frequency of 7 Hz evoked independently of the temporal structure of the stimulus (top inset). The axes labels of all insets are the same as in *A*.

we hypothesize that they may reflect a new mapping rule in M1: the separate mapping of different modes of motor control.

The control and interaction of rhythmic and nonrhythmic whisking

Long stimulus patterns at high frequencies, as used the present study, have been shown to elicit a strong and continuous inhibi-

tion in cells residing within a large area surrounding the electrode, only interrupted by single (or few) action potentials after each stimulus pulse (Chung and Ferster, 1998; Butovas and Schwarz, 2003). Despite these effects that should entirely disrupt local rhythmic activity in M1 at 5–12 Hz, whisking in that frequency range could be elicited in awake rats, indicating that the oscillator generating the whisking rhythm must reside outside RW. Our data, at least for the action of RW, strongly support previous evidence for the intercalation of a CPG (Welker, 1964; Lovick, 1972; Semba and Komisaruk, 1984; Gao et al., 2001) most likely located in the brainstem (Hattox et al., 2002, 2003).

Long duration of stimulation was the decisive measure that allowed us to elicit rhythmic whisking from RW. It could be argued that long stimulation is needed to evoke rhythmic movements because extended neural structures of the CPG have to be recruited. However, the short latency with which whisking commenced after stimulus onset does not support this argument. Rather, extended duration of stimulation was needed to allow the movement to reveal its cyclic organization. This feature sets whisking apart from other (presumably) CPG-controlled movements. Rhythmic jaw movements in monkeys and rats were reported to start only after several hundreds of milliseconds after the onset of long stimulus trains (Moriyama, 1987; Huang et al., 1989). Rhythmic stepping may not be evoked *de novo* by long cortical stimulation at all, as judged from the absence of reports on this issue. An explanation for these differences may be that the feasibility to trigger rhythmic movement and their onset latency may be related to the complexity of the movement or the intricacy of the organization of the CPG, respectively. It is conceivable that whisking, which just needs the control of two antagonistic sets of muscles (Berg and Kleinfeld, 2003b), uses a CPG of much simpler structure than mastication or locomotion depending on synergies of far ranging sets of muscles.

The present results are the first to point specifically to possible contributions of M1 to explorative whisking that go beyond rhythm generation or mere triggering of the movement. It is known that rats vary whisking frequency as well as changes in the set point dependent on the perceptual task at hand, most likely to optimize performance (Carvell and Simons, 1995). Our double-electrode stimulations suggest that M1 may control these adaptive features of cyclic whisking. First, application of synchronous stimulus pattern to two electrodes in RW resulted in an increment of the whisking frequency compared with the effect of stimulation via only one electrode. Second, joint activation of RW and RF could be used to change the set point of whisking because double stimulation from both subregions shifted rhythmic whisking backward. Although the present results using multielectrode stimulation may provide a useful framework of what might be possible, it will be necessary to confirm the significance of these suggested patterns by electrophysiological recordings during discriminative whisking.

Nonrhythmic movements from RF in awake animals elicited exclusively retraction movements. However, rats are clearly capable to generate protraction movements (Bermejo and Zeigler, 2000). A possible explanation for this puzzling finding comes from previous results indicating that intrinsic muscles (pulling forward) could be activated by electrical stimulation but that the joint activation of the stronger extrinsic muscles (pulling backward) outweighs the action of the intrinsic muscles, resulting in a net backward movement (Berg and Kleinfeld, 2003a). The fact that muscles pulling the whiskers forward are readily activated from RF leaves the possibility that RF may generate protraction movements when activated naturally. One possibility is that re-

traction and protraction may be spatially represented in an intermingled way (i.e., by individual neurons or by very small groups of neurons that cannot be selectively activated by microstimulation). In line with this hypothesis is the recent finding that direction selectivity in M1 arm representation in monkey is organized on a subcolumnar range (Amirikian and Georgopoulos, 2003). Another possibility is that the two movement directions may be represented by the population rate within the network. Indeed, whole-cell recordings *in vivo* have demonstrated such a mechanism even on the level of individual neurons (Brecht et al., 2004). In view of our previous finding that horizontal activation falls monotonically with distance from the stimulation electrode (Butovas and Schwarz, 2003), this latter scheme would predict that rhythmic whisking with retracted set points should be found closer to RF, whereas the sites featuring protracted set points should be found further away. Although detailed mapping inside RW has to be performed with electrode arrays of far higher resolution than the ones used here in future experiments, our data show a bias toward retracted set points, tentatively supporting this prediction.

Mapping of control mode

The principal difference between the two subregions investigated here was that they were associated to different sets of relevant instances within the motor system. First, movement evoked from RW invoked the activity of an oscillator at comparatively high threshold intensities, whereas the movement evoked from RF was nonrhythmic and was observed with very low current amplitudes. Second, retraction evoked from RF was accompanied by movements of the eyes, eye lids, ears, and snout (we also obtained circumstantial evidence for head movements evoked from RF after release of the head fixation), whereas such a complex context was absent during evoked rhythmic whisking from RW. If, as has been argued (Graziano et al., 2002), long stimulation reveals sets of functional associations of the cortical site stimulated, then we may conclude that RW and RF, in principle, subserve movements that use different types of control and are performed in different behavioral contexts. In this way of thinking, the close association of face movements to whisker retraction could be, in principle, part of defensive or aggressive behavior. Which of the two correlates of the control mode discussed so far, association to distinct behavior or association to distinct subcortical connectivity, is the defining instance for the mode of control and demands separated representation? The facility to evoke combined movements from the two subregions suggests that the subregions may, in principle, act concertedly (e.g., to set point adjustments as discussed above). In this case, the two control modes would be used in a common behavioral context. It is feasible that long stimulation uncovers just the strongest of all possible associations (laid down, for example, by the strongest intracortical connections). In this sense, the face movements seen with retraction could be the tip of the iceberg, leaving many weaker but still possible associations undetected. From these considerations, it seems likely that it is separate subcortical signaling pathways rather than behavioral contexts that demand spatial separation.

Is the presumptive mapping of modes of control a special case in rat wM1, or could it be a general rule valid also for other representations in M1? A closer look to the mammalian motor system suggests that this question is reasonable, because the use of the same motor periphery using presumptively different access routes is the rule rather than an exception. For instance, most of so-called “semiautomatic” movements (e.g., defense, righting, licking, mastication, and locomotion) are speculated to use CPGs

downstream from M1 (Orlovsky, 1972; Lund and Lamarre, 1974; Armstrong and Drew, 1984; Moriyama, 1987; Huang et al., 1989; Zhang and Sasamoto, 1990; Rho et al., 1999; Cooke and Graziano, 2004). However, the very same body parts involved in semi-automatic movements can be moved in a nonautomatic, “arbitrary” way, most likely using different signaling routes. The benefit of segregated representations of movements that require different access routes to the muscles might be that separated neuronal structures can be devoted to quite different computational tasks. In doing this, unwanted interferences between the two modes of control are reduced, and computational problems during separated activation of the two modes are avoided. In this sense, the separation of nonrhythmic retraction movements and rhythmic whisking may reflect a principal strategy governing the organization of the map of M1: flexibility of movement performance is traded for gains in computational speed and simplicity (Schieber, 2001). We therefore hold it likely that detailed remapping studies, preferentially using long stimulation trains, may unearth segregation of modes of control also in other M1 regions and species.

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