

Whisker movements evoked by stimulation of single pyramidal cells in rat motor cortex

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Neuronal activity in the motor cortex is understood to be correlated with movements, but the impact of action potentials (APs) in single cortical neurons on the generation of movement has not been fully determined. Here we show that trains of APs in single pyramidal cells of rat motor cortex can evoke long sequences of small whisker movements. For layer-5 pyramids, we find that evoked rhythmic movements have a constant phase relative to the AP train, indicating that single layer-5 pyramids can reset the rhythm of whisker movements. Action potentials evoked in layer-6 pyramids can generate bursts of rhythmic whisking, with a variable phase of movements relative to the AP train. An increasing number of APs decreases the latency to onset of movement, whereas AP frequency determines movement direction and amplitude. We find that the efficacy of cortical APs in evoking whisker movements is not dependent on background cortical activity and is greatly enhanced in waking rats. We conclude that in vibrissae motor cortex sparse AP activity can evoke movements.

In mammals, a key brain structure in motor control is the primary motor cortex (M1), a large frontal cortical area^{1–3}. The role of M1 neurons in generating movements has been explored by extracellular stimulation with small currents (intracortical microstimulation)⁴ and by extracellular recordings of single neurons^{5,6}.

Extracellular stimulation experiments in M1 have shown that there are very low current thresholds (as low as 1–2 μ A) for movement initiation³ that are thought to stimulate few cortical neurons^{7,8}. Extracellular recordings from single cells in M1 of awake, behaving monkeys have also shown that there is a close match between AP activity and movement⁶. The quantitative analysis of such recordings by an AP population vector^{9,10} and by real-time analysis of AP activity in M1—motivated by the desire to construct cortically controlled neuroprosthetic devices^{11–14}—has led to the conclusion that the activity of only a few hundred to a few tens of neurons is sufficient to specify accurately complex limb movements.

The relationship between neuronal and muscular activity has been also analysed by computing averages of muscle activity (assayed by electromyographic recordings) triggered relative to neuronal APs¹⁵. This technique has revealed significant correlations between cortical single-cell activity and muscle activity^{15–17}. Thus, extracellular stimulation and recording studies indicate that there is a close correlation between activity in the motor cortex and muscular activity, consistent with the known synaptic connectivity between cortex and motoneurons¹⁶. Intracellular stimulation of cortical cells evokes changes in electromyographic recordings¹⁸ and might allow a more direct investigation of the relationship between cortical APs and movement.

In rats, whisker movements can escape from sleep or anaesthesia-induced paralysis^{19,20}. Such movements are rhythmic, are restricted to the plane of the whisker row, and can be quantified as a simple back-and-forth movement^{21–24}. The movements of individual whiskers can be tracked readily, making them a convenient system to quantitatively study the generation of motor output²⁵. Here we have used whole-cell recordings to examine the motor effects of intracellularly evoked APs in identified layer-5 (L5) and layer-6 (L6) pyramidal cells in the vibrissae motor cortex²⁶ of anaesthetized rats.

Movements evoked by intracellular stimulation

Figure 1 shows a pyramidal L6 cell (Fig. 1a) in which intracellular current injection evoked 10 APs at 50 Hz (Fig. 1c). This stimulation caused backward movements of whisker E1 and adjacent whiskers (Fig. 1b and Supplementary Movie 1), which were the same whiskers that were deflected backwards by extracellular stimulation in the same region (Fig. 1d). Movements consisted of bouts of backwards-and-forwards sweeps, which we refer to as a ‘whisking burst’, beginning after a variable latency of 0.05–2.0 s. The peak of the evoked movement was delayed (0.3 s to 50% of the amplitude) and the movement lasted for several seconds (Fig. 1e). We quantified the peak-to-peak amplitude of movements in the second immediately after current injection and in the second just before current injection. Action potential initiation evoked significant movements ($2.12 \pm 0.17^\circ$ poststimulation amplitude versus $1.62 \pm 0.14^\circ$ prestimulation amplitude; paired *t*-test, $P < 0.05$) but injection of hyperpolarizing currents did not (paired *t*-test, $P > 0.3$; Fig. 1e), resulting in significantly larger ratios of post- to prestimulation amplitudes for AP initiation than for hyperpolarizing current injection (paired *t*-test, $P < 0.05$).

To assess the significance of movement effects in individual experiments, we measured the movement responses trial by trial across 10–30 stimulations. Peak-to-peak amplitudes of whisker movements in the second just after initiation of APs were compared with peak-to-peak amplitudes of spontaneous movements in the second immediately before stimulation. A significant poststimulation increase in whisker movements was detected in 9 out of 44 regularly spiking or intrinsically bursting neurons (two-tailed, paired *t*-tests, $P < 0.05$). In addition, we analysed the degree to which averaged movement records in the first second after AP initiation ($n = 44$ cells) differed in peak-to-peak amplitude from movements occurring in the second preceding stimulation. This measurement showed that, across cells, poststimulation changes in average whisker position were significantly larger than prestimulation changes (ratio of post- to prestimulation amplitudes, 1.30 ± 0.09 ; paired *t*-test, $P < 0.01$; Fig. 2a).

In control experiments, 5-nA current steps applied through patch pipettes to the extracellular space of M1 (threefold larger than those

applied in intracellular stimulation (1.68 ± 0.8 -nA steps)) did not evoke significant whisker movements (ratio of post- to prestimulation amplitudes, 1.03 ± 0.13 ($n = 10$); Fig. 2b). Whereas AP-evoked movements were clearly stimulus locked (Fig. 2c), even the largest poststimulation deflections observed with extracellular stimulation were not (Fig. 2d). Similarly, in 13 cells hyperpolarizing currents did not evoke whisker movements (ratio of post- to prestimulation amplitudes, 1.14 ± 0.14), whereas APs evoked by depolarizing currents did (ratio of post- to prestimulation amplitudes, 1.49 ± 0.17 ; Fig. 2a, c). In five neurons of L5 and L6 of the primary visual cortex, intracellularly evoked APs did not evoke whisker movements (ratio of post- to prestimulation amplitudes, 1.02 ± 0.24 ; Fig. 2b). Thus, in none of the 31 individual control experiments with extracellular, hyperpolarizing, subthreshold current injection or AP stimulation of visual cortex neurons did we observe significant effects on movement. Prestimulation and poststimulation amplitudes did not differ between control experiments (paired t -test, $P > 0.22$), and the ratio of poststimulation to prestimulation amplitudes was significantly larger in AP initiation experiments (unpaired t -test, $P < 0.04$). Thus, the control experiments indicate that AP trains in single, deep-layer pyramidal cells in M1 cause whisker movement.

Effects of intra- and extracellular stimulation

Whisker movements evoked by intracellular stimulation were small in amplitude: most were less than 1° , and the largest were $2\text{--}3^\circ$ after averaging. In weakly effective cells, the effect of intracellular stimulation was restricted to modulation of the ongoing whisker movements, whereas in the most effective cells stimulus-locked movements were evoked in most of the trials. Evoked movements had long latencies (minimum ~ 50 ms, but often longer) and were complex in that they always involved several whiskers and usually consisted of second-long sequences of movements (Supplementary Movies 1 and 2). The rhythmicity of evoked movements was always the same as that of ongoing movements and varied with the depth of anaesthesia.

Extracellular stimulation, at current levels just above threshold ($32 \pm 22 \mu\text{A}$), evoked short-latency (<100 ms), small-amplitude ($<5^\circ$) whisker movements. Movements evoked by either intracellular stimulation of pyramidal neurons or extracellular stimulation shared the same topography of deflected whiskers and initial direction of deflection (in 85% of cases).

Movements evoked by extracellular stimulation had a shorter latency, were more stringently locked to stimulation onset, and had a shorter duration (usually <0.5 s; Fig. 1d, e) than did movements

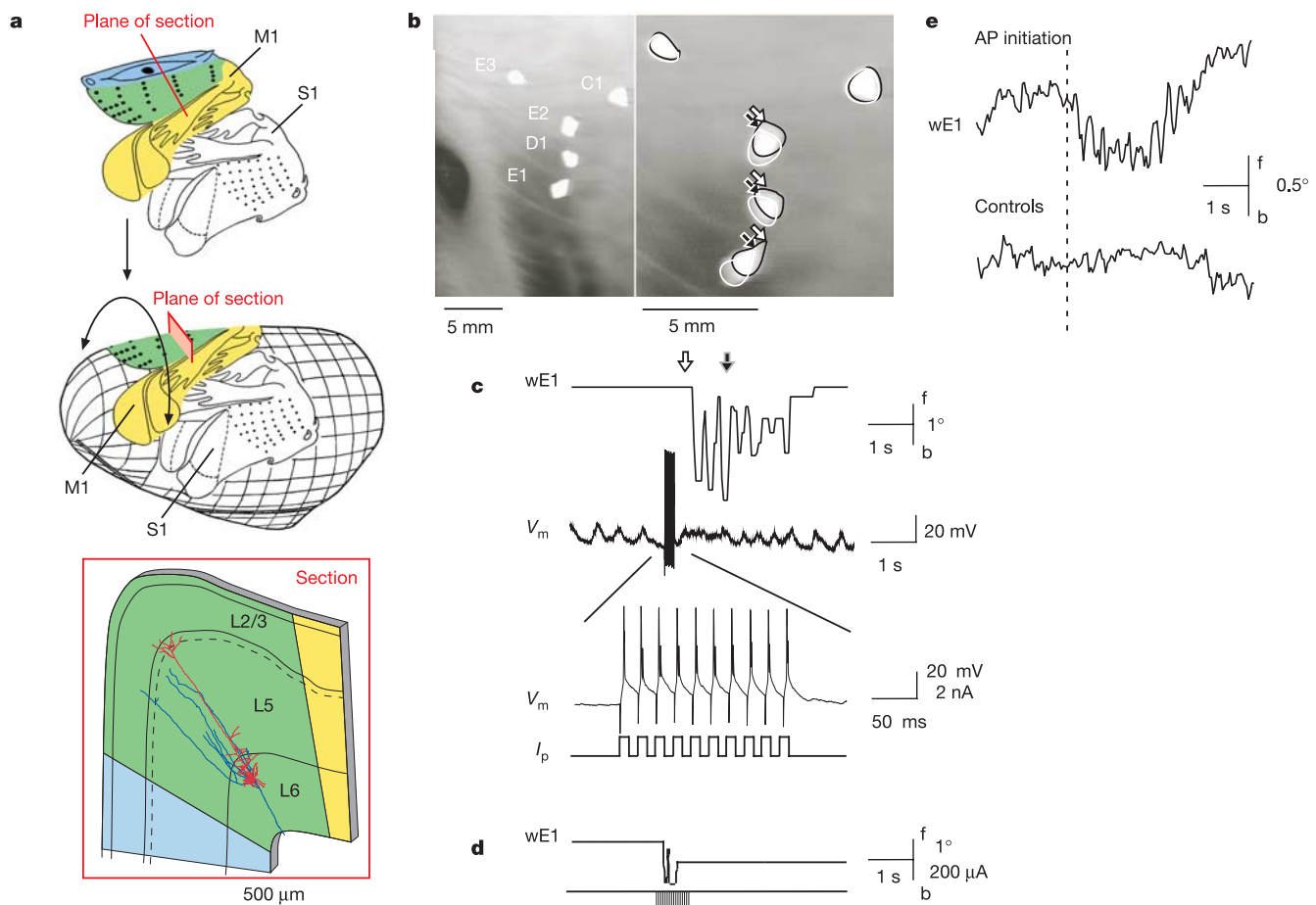


Figure 1 Whisker movements evoked by intracellular stimulation of an L6 pyramidal neuron. **a**, Top, flattened maps of the primary motor (M1) and somatosensory (S1) cortices. Middle, dorsolateral view of a rat cortical hemisphere, indicating S1 and M1. Bottom, coronal section through M1, indicating the dendrites (red) and axon (blue) of the stimulated L6 pyramidal neuron. Blue, area Cg1 (eye movement motor cortex); green, area AGm (vibrissae motor cortex); yellow, area AGl (somatic motor cortex). **b**, Left, top view of rat head and foil-labelled (bright spots) whiskers. Right, superimposed images of prestimulation (black outlines) and poststimulation (white outlines) whisker positions

(arrows). **c**, Top trace, position of whisker E1 (wE1) in an intracellular stimulation trial (10 APs at 50 Hz). Arrows indicate the time points at which the images shown in **b** were taken. Bottom traces, membrane potential recordings and current injection steps. f, forward movement; b, backward movement. **d**, Position of whisker E1 in an extracellular stimulation trial (top) and the stimulation pattern (bottom). **e**, Movement averages of 30 single-cell stimulation trials (top) and 30 control trials (bottom) with hyperpolarizing current steps. Broken line indicates the onset of current injection.

evoked by intracellular stimulation. Unexpectedly, although single or dual whisker movements were regularly evoked by extracellular stimulation (at ~25% of sites, 14 out of 55 experiments), we never observed single or dual whisker movements after intracellular stimulation. Thus, at threshold levels, extracellular stimulation can have smaller movement fields than can intracellular stimulation.

Patterning of whisker movements is layer-specific

To understand how evoked whisker movements are related to the laminar position of motor cortex cells, we analysed movement patterns evoked by L5 ($n = 12$) and L6 ($n = 11$) pyramidal cells, in which we had observed poststimulation movements that were larger than prestimulation movements (ratio of post- to prestimulation amplitudes, >1.2). We found that stimulation of an L5 pyramidal cell (Fig. 3a) evoked movements in which the timing of an individual forwards-and-backwards whisking movement relative to the stimulation AP train was similar from one trial to the next (Fig. 3b and Supplementary Movie 2). We refer to this movement as whisker movements with a ‘constant phase relative to stimulation’. As a consequence, after averaging the movement records, modulation at the whisking frequency became apparent (Fig. 3c). The power spectra of both the individual trial and the averaged movement are characterized by a peak at 3.5 Hz, which corresponds to the

whisking frequency (data not shown). In the population average, we also observed modulation of the evoked movement at the whisking frequency ($n = 12$; Supplementary Fig. 1a).

Stimulation of an L6 cell (Fig. 3d) evoked rhythmic whisker movements in which the timing of an individual forwards-and-backwards movement varied from one trial to the next (Fig. 3e and Supplementary Movie 1). Thus, the L6-evoked whisker movements had a ‘variable phase relative to stimulation’, and the periodic modulation at the whisking frequency averaged out across trials (Fig. 3f). The average of L6-evoked movements is described by the outline (‘envelope’) of one or several whisking bursts (Fig. 3f). The power spectrum of the average movement revealed that only a single, low-frequency (<0.5 Hz) peak was present at frequencies that were contained in the envelope of a whisking burst. The same observations were made for the population average ($n = 11$; Supplementary Fig. 1b). Averaged movements evoked by L5 and L6 cell stimulation were significantly different in peak power frequency (unpaired t -test, $P < 0.001$), whereas the peak power frequencies of individual trials were not (unpaired t -test, $P > 0.2$; Supplementary Fig. 1c). Thus, across trials the whisking rhythm is stable when L5 cells are stimulated, but averages out when L6 cells are stimulated.

Effects of AP frequency and number

To explore how AP frequency in M1 pyramidal cells influences movements, we studied whisker movements evoked by trains of 10 APs, initiated at rates of 10, 50 or 100 Hz. In an L6 pyramidal cell (Fig. 4a), 10 APs at 50 or 100 Hz evoked a sequence of bursts of

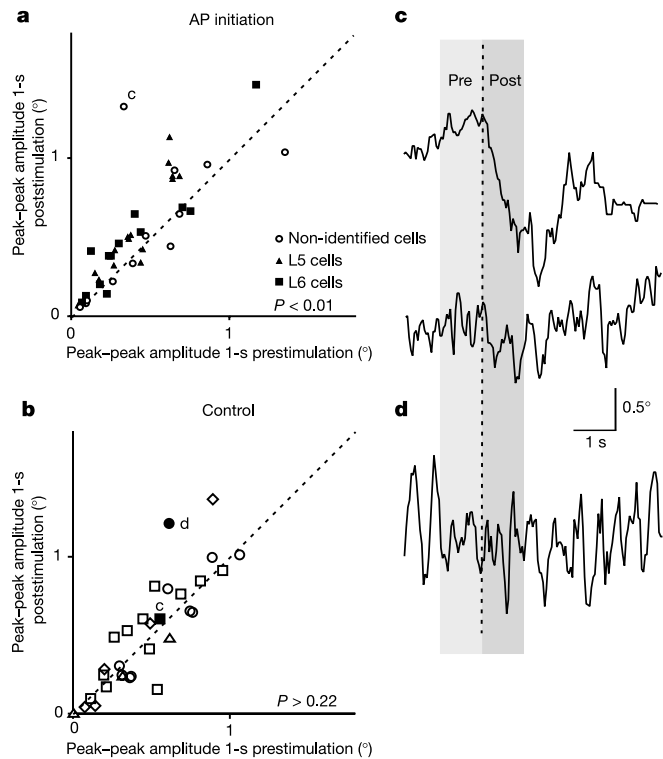


Figure 2 Action potential initiation specifically evokes whisker movements. **a**, Pre- and poststimulation amplitudes of whisker movement during single-cell stimulation in 44 neurons. Data are based on averaged movements (10–30 trials) evoked by stimulation with 10 APs at either 50 Hz (26 cells) or 100 Hz (18 cells). **b**, Pre- and poststimulation amplitudes of whisker movement in control trials. Circles, extracellular injection of 5-nA current steps; squares, intracellular injection of hyperpolarizing current steps; triangles, intracellular injection of depolarizing but subthreshold current steps; diamonds, APs in neurons of primary visual cortex. **c**, Movement trace of the largest evoked movement (top) and the respective control trace with hyperpolarizing current injection (bottom). **d**, Largest (random) movement effect observed under control conditions. Broken lines in **c** and **d** indicate the onset of stimulation. The time intervals used for determining the movement amplitudes shown in **a** and **b** are indicated by grey shading in **c** and **d**.

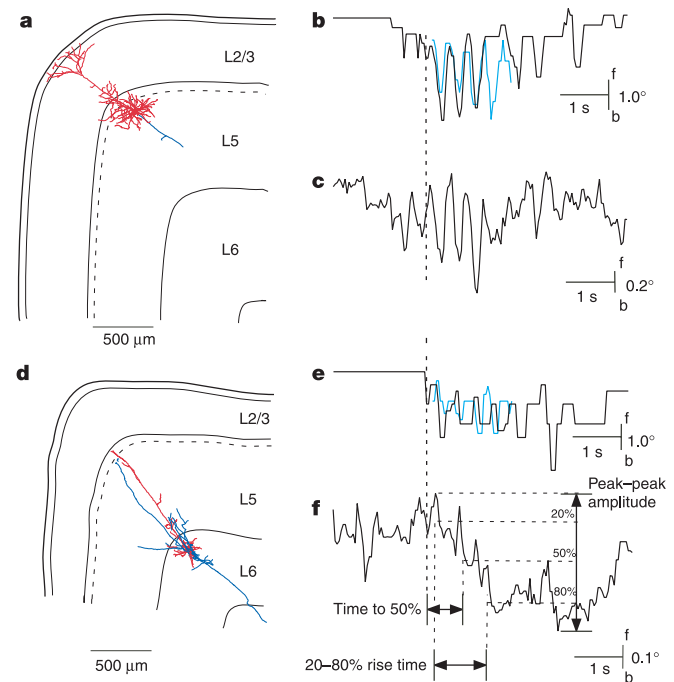


Figure 3 Whisker movements evoked by intracellular stimulation of single L5 and L6 cells. **a**, Dendrites (red) and axon (blue) of the stimulated L5 neuron. Proximal extracellular stimulation evoked backward movement of a single whisker (D1). **b**, Position of whisker D1 in a partially (blue) and fully (black) shown intracellular stimulation trial (10 APs at 50 Hz). Note that the evoked movements are in phase. **c**, Movement average (15 trials). **d**, Dendrites (red) and axon (blue) of the stimulated L6 neuron. Proximal extracellular stimulation evoked backwards movements of several whisker rows, with maximal deflection of whisker C1. **e**, Position of whisker C1 in a partially (blue) and fully (black) shown intracellular stimulation trial (10 APs at 50 Hz). Note that the evoked movements are not in phase. **f**, Movement average (15 trials). Movement parameters were determined as indicated. Broken lines in **b**, **c**, **e** and **f** indicate the onset of AP initiation.

backwards whisking movements. In the averaged movement (Fig. 4b), the outlines (envelopes) of two such bursts are apparent: the amplitudes of the evoked backwards movements were substantially larger at 100-Hz than at 50-Hz stimulation. Action potentials

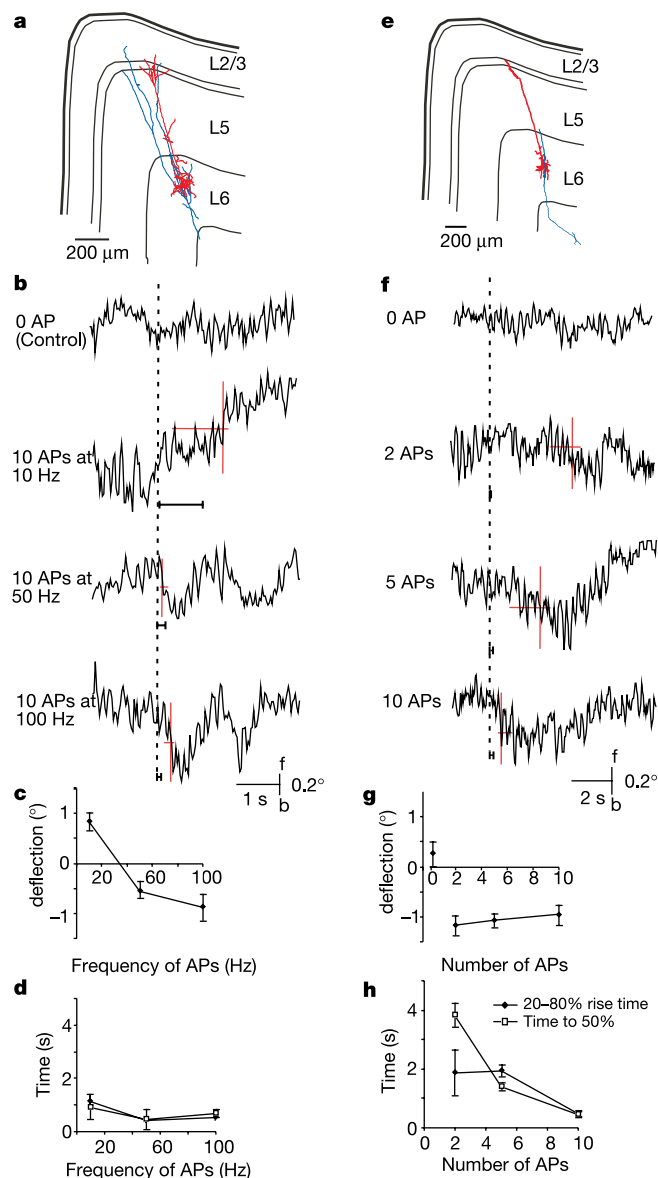


Figure 4 Effect of AP number and frequency on evoked movements. **a**, Dendrites (red) and axon (blue) of the stimulated L6 neuron. Proximal extracellular stimulation evoked backwards movements of C-row whiskers, with maximal deflection of whisker C2. **b**, Average movements of whisker C2 in 15 stimulation trials with initiation of 10 APs at 10, 50 or 100 Hz. Vertical red lines indicate the 50% amplitude time point and the amplitude measured; horizontal red lines indicate the 20–80% rise times. **c**, Population data for the effect of AP frequency on mean deflection amplitudes. Data are from seven neurons (three L5 pyramidal cells, three L6 pyramidal cells and one unidentified regularly spiking cell) located at sites where extracellular stimulation evoked backwards movements. **d**, Effect of AP frequency on movement dynamics (same cells as in **c**). **e**, Dendrites (red) and axon (blue) of the stimulated L6 neuron. Proximal extracellular stimulation evoked backwards movements of posterior whiskers in rows C, D and E, with maximal deflection of whisker E1. **f**, Average movements of whisker E1 in 12 intracellular stimulation trials with initiation of 2, 5 or 10 APs at 50 Hz. Red lines are the same as in **b**. **g**, Population data for the effect of AP number on mean deflection amplitudes. Data are from nine neurons (two L5 pyramidal cells, four L6 pyramidal cells and three unidentified regularly spiking cells) located at sites where extracellular stimulation evoked backwards movements. **h**, Effect of AP number on movement dynamics (same cells as in **g**). Broken lines in **b** and **f** indicate the onset of AP initiation.

at 10 Hz resulted in a marked, slow, forwards movement after the train. Six additional experiments gave the same result (Fig. 4c, d and Supplementary Fig. 2a).

The effect of AP frequency on the peak-to-peak amplitude was significant (analysis of variance (ANOVA), $F_{(2,6)} = 12.18$; $P < 0.003$), whereas effects on the 20–80% rise time (ANOVA, $F_{(2,6)} = 0.9$; $P > 0.4$) and the time to 50% amplitude (ANOVA, $F_{(2,6)} = 0.34$; $P > 0.7$) were insignificant. The effects of variation of AP frequency were similar for L5 and L6 cells. Consistent with the results from intracellular stimulation, we observed a reversal of whisker movement direction at frequencies of less than 50 Hz for extracellular stimulation (data not shown).

To investigate the effect of AP number, we carried out experiments in which 2, 5 or 10 APs were evoked at a rate of 50 Hz in an L6 cell (Fig. 4e, f). With an increasing number of APs, the latency and rise time of the evoked backwards movements decreased

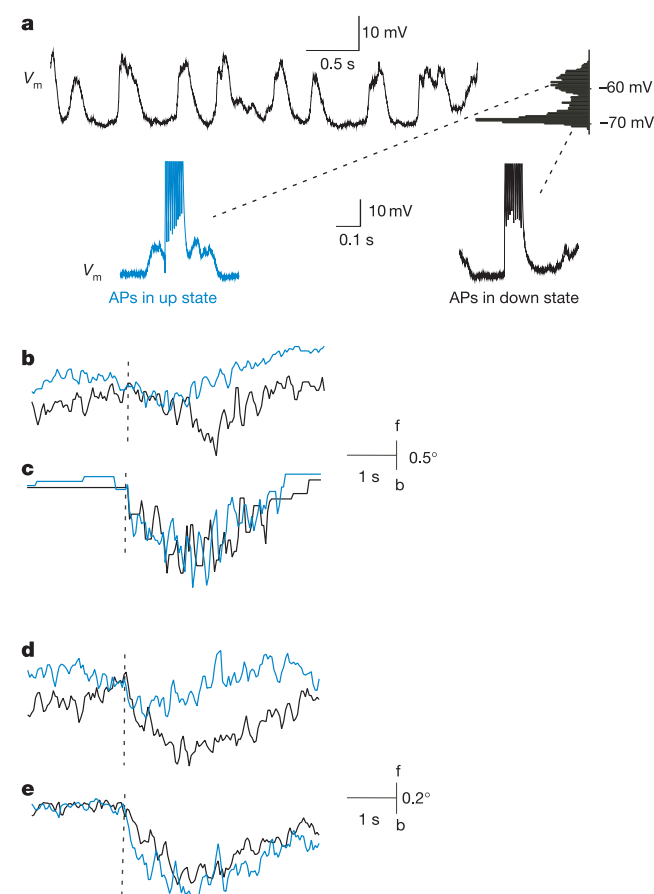


Figure 5 Efficacy of APs initiated in cortical up states and down states with and without prestimulation movement. **a**, Top, the membrane potential of an L6 cell (same cell as in Fig. 4e) fluctuated between more depolarized (up states) and less depolarized (down states), leading to a bimodal distribution of membrane potential values over time (histogram on right). Bottom, AP initiation (10 APs at 100 Hz) in up states (left, blue) or down states (right, black); APs have been clipped. **b**, Average evoked movements for APs initiated in up states (blue, $n = 8$ trials) or down states (black, $n = 12$ trials). **c**, Average evoked movements with AP initiation in down states (black, $n = 3$ trials) or up states (blue, $n = 6$ trials) for stimulation trials without prestimulation movements. **d**, Population average (133 trials, $n = 9$ cells in which large backwards movements were evoked (three L5 pyramidal cells, three L6 pyramidal cells and three unidentified regularly spiking cells)) of movements evoked by AP initiation in down states (black trace) or up states (blue trace). **e**, Population average (56 trials, $n = 9$ cells) of movements evoked by AP initiation in down states (black trace) or up states (blue trace), when trials with prestimulation movements were excluded. Broken lines in **b–e** indicate the onset of AP initiation.

progressively (Fig. 4f). The amplitude remained either constant or increased slightly. Eight additional experiments gave the same result (Fig. 4g, h and Supplementary Fig. 2b). The time course of movements evoked by fewer than 10 APs was very slow. Because the motor effects of initiating 1 AP (data not shown) and 2 APs were variable from cell to cell and from trial to trial, further work will be required to determine the robustness of the effects of single APs on movements.

The effect of AP number (for 1, 2, 5 or 10 APs) on the time to 50% amplitude was more pronounced than the effect of AP number on the 20–80% rise time (Fig. 4h), indicating that AP number affects latency more than rise time. An increasing AP number significantly decreased the 20–80% rise time (ANOVA, $F_{(3,8)} = 9.18$; $P < 0.02$) and the time to 50% amplitude (ANOVA, $F_{(3,8)} = 8.18$; $P < 0.01$), but had no significant effect on the peak-to-peak amplitude (ANOVA, $F_{(3,8)} = 0.42$; $P > 0.4$). The increase of movement latency with decreasing AP number was observed in both L5 and L6 cells.

Interaction of APs with ongoing cortical activity

Under the anaesthesia used in this study, the membrane potential of M1 neurons fluctuates by 10–15 mV between depolarized (up states) and hyperpolarized (down states) membrane potentials (Fig. 5a). Up states and down states reflect synchronous large-amplitude fluctuations of cortical population activity^{27–29}. To understand how the motor effects of single-cell discharges depend on the ongoing cortical activity, we sorted data into stimulation trials in which APs were initiated either in up states (Fig. 5a, bottom, blue trace) or down states (Fig. 5a, bottom, black trace). Down-state AP initiation evoked larger movements (1.3°; Fig. 5b, black trace) than did up-state AP initiation (0.5°; Fig. 5b, blue trace). The pattern of cortical up states and down states was closely related to the ongoing whisker movements of the rat, with whisker movements occurring preferentially in prolonged up states. Thus,

movements evoked by APs in the up state sometimes counteracted the trajectory of ongoing movements.

To minimize the effects of ‘spontaneous’ movements associated with up states and down states, we carried out an analysis in which we excluded stimulation trials with spontaneous (prestimulation) movement (Fig. 5c). For this subset of data, down-state AP initiation (2.0°; Fig. 5c, black trace) and up-state AP initiation (1.7°; Fig. 5c, blue trace) led to very similar evoked movements. At the population level, AP initiation in up states (0.35°; Fig. 5d, blue trace) evoked smaller movements than did AP initiation in down states (1.05°; Fig. 5d, black trace), but, in the absence of prestimulation movements, AP efficacy in up and down states was similar (1.31° versus 1.15°; Fig. 5e).

Thus, APs initiated in up states are seemingly less effective only because of the effects of ongoing movement patterns associated with cortical up and down states. This view was supported when we corrected evoked movements by subtracting the average movement patterns associated with up states and down states (Supplementary Fig. 3). Thus, much of the variability in the single-cell stimulation effects might be attributed to fluctuations in ongoing cortical population activity and associated movements.

AP trains are more effective in awake rats

We observed progressively larger evoked whisker movements with a decreasing depth of anaesthesia (data not shown; see also refs 7, 26, 30, 31). This effect was studied more closely in four cells that we stimulated under conditions of both anaesthesia and wakefulness. In one L6 cell (Fig. 6a), no movement was evoked by intracellular stimulation (10 APs at 100 Hz) under anaesthesia (Fig. 6b), but a very large whisker movement could be evoked by the same stimulation in the awake rat (Fig. 6c). In this restricted sample, whisker movements were evoked by AP initiation in the awake condition (mean ratio of post- to prestimulation amplitudes, 2.2 ± 0.7), but not under the anaesthetized condition (ratio of

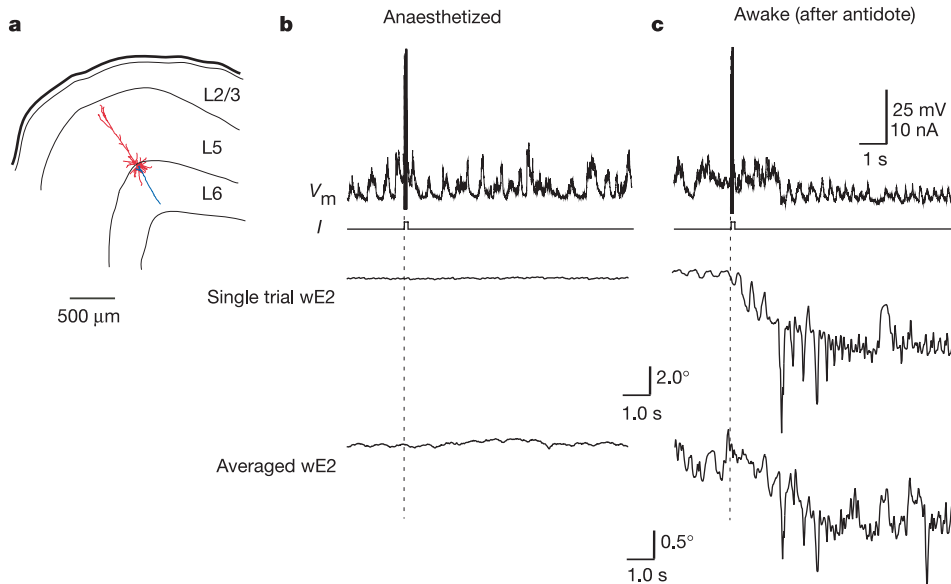


Figure 6 Cortical APs are more effective in awake rats. **a**, Dendrites (red) and axon (blue) of the stimulated L6 neuron. Proximal extracellular stimulation evoked backwards movement of several whisker rows, with maximal deflection of whisker E2 (wE2). **b**, Intracellular stimulation in a head-fixed rat anaesthetized with a mixture of medetomidine, midazolam and fentanyl. Top to bottom, the four traces show membrane potential, the stimulation current, position of whisker E2 during a single stimulation (10 APs at 100 Hz) trial, and average position of whisker E2 for ten stimulation trials. **c**, Stimulation of the same cell after anaesthesia was antagonized with a mixture of

atipamezol, flumazenil and naloxone. A local field potential recording indicated a marked change in the pattern of population activity (not shown). The top three traces indicate the last stimulation trial, the bottom trace indicates the averaged movement trace of the last ten stimulation trials (~4 min after antagonizing anaesthesia) before the recording was disrupted owing to a rapid body movement of the rat. Seconds later, head fixation was discontinued and the rat began to explore the recording set-up. Conventions are the same as in **b**.

post- to prestimulation amplitudes, 0.94 ± 0.08).

For the four cells stimulated in awake rats, we observed a larger evoked slow whisker movement (Fig. 6c) and a larger evoked fast whisker movement (data not shown) than in any of the 44 cells stimulated in anaesthetized rats. These data, albeit restricted to a small sample, indicate that anaesthesia dampens the movement effects and that AP initiation in the vibrissae motor cortex of awake rats is highly effective.

Discussion

We have shown that a train of APs in a single pyramidal cell of rat M1 can cause whisker movement, thereby contradicting the view that “the discharge of an individual pyramidal neuron in M1 is meaningless”³². Three results support our conclusion: first, currents applied intracellularly were about four orders of magnitude smaller (nanoamperes versus tens of microamperes) than for extracellular stimulation, making it unlikely that unintended stimulation through an extracellular pathway contaminated the results; second, the patterning of single-cell-evoked movements was dependent on the layer in which the stimulated cell was located; and third, evoked movements varied with the number and frequency of initiated AP trains.

We observed complex and prolonged movement patterns that involved repeated movement of several whiskers. Thus, in the vibrissae motor cortex, individual pyramidal cells seem to specify motor programs rather than single muscle contractions. It seems that the lamination of M1 mirrors specific properties of whisking, such as the timing of the individual whisking movement by L5 activity and the grouping of multiple whisking movements in bursts of whisking by L6 activity. Extracellular single-unit recordings have described AP activity in deep-layer M1 cells that was correlated with the initiation of whisker movements but not with the phase of whisking³³, consistent with our observations on single-cell stimulation effects in L6. A laminar difference in stimulation effects has been also indicated by extracellular stimulation experiments in cats³⁴, in which stimulation of deeper layers evoked more prolonged and less phasic contractions of limb muscles than did more superficial stimulation.

Movements evoked by intracellular stimulation occur with long latency and greatly outlast the duration of the AP train. The direction of movements is dependent on the stimulation frequency. These observations are indicative of indirect control of whisker movements by neurons of the vibrissae motor cortex. Indeed, cells in M1 do not directly project to the facial nucleus, which innervates the whisker musculature³⁵, but reach the facial nucleus through disynaptic pathways. A principal pathway consists of a projection from M1 L5 cells to an ensemble of cells distributed around the facial nucleus, which are thought to form a ‘central pattern generator’ for whisking movements^{35–37}. The complexity of the evoked movements might result from activation of the putative pattern generator. Our data indicate that L5 cells do not merely gate an otherwise freely running pattern generator. Instead, APs in single L5 cells can either reset or initiate the whisking rhythm. L6 neurons project to the thalamus³⁸, and the bursts of whisking movements after L6 cell stimulation might indicate that thalamic circuits are involved in the grouping of individual protractions and/or retractions into bursts of whisking.

An intriguing issue is how a small number of APs evoked in a single neuron can be so effective in the extensive M1 network. It might be that our whole-cell recordings tended to sample a subset of neurons—for example, particularly large neurons—that might be unusually effective in evoking movements. However, we have no evidence for such a sampling bias. Initiation of multiple APs might recruit both intracortical and subcortical postsynaptic target neurons by temporal summation³⁹ and by synaptic facilitation, which is a prominent feature of corticofugal output synapses⁴⁰. On the basis of its surface area, we estimate that the agranular medial area, from

which whisker movements can be evoked, contains 1–1.5 million neurons⁴¹. The largest whisker movements made by rats have amplitudes of around 100° , whereas single-cell stimulation in anaesthetized rats often evoked multiwhisker movements with an amplitude of around 0.5° . Thus, it seems possible that the activity of only a few hundred neurons could be sufficient to achieve the full set of whisker movements.

One fact that might contribute to the effectiveness of single-cell activity is the low rate of spontaneous APs in cortical neurons^{42–45}. Movements evoked by single cells seem to vary as a result of variations in ongoing cortical activity and the associated movements. Taking this source of variance into account, our data indicate that there is an unanticipated degree of precision and sensitivity in the mechanisms that ‘translate’ APs into movements. We conclude that, in vibrissae motor cortex, sparse cortical activity is sufficient to generate motor output. □

Methods

Rats

We used standard surgical and electrophysiological techniques⁴⁵. Wistar rats ($n = 63$) were anaesthetized with ketamine hydrochloride (90 mg per kg (body weight) intraperitoneally) and xylazine (5 mg per kg intraperitoneally) for surgery. Pressure points and skin incisions were infused with lidocaine, and rats received supplemental doses of ketamine (20 mg per kg) and acepromazine (0.02 mg per kg, intramuscularly) as needed. Vibrissae motor cortex (A1–A2.5 and L1–L2; see Supplementary Information) and, in control trials, visual cortex (P5 and L3.5) were exposed. All experimental procedures were carried out in accordance with the animal welfare guidelines of the Max Planck Society.

Stimulation and recordings

Extracellular stimulation (300-ms trains of 100 monophasic, negative, 0.3-ms stimulation pulses) was delivered through a pipette (tip resistance 0.5–1 M Ω) at 1,500 μ m below the pial surface. Intracellular stimulation consisted of 5-ms or 10-ms current steps for evoking individual APs in sequences, as specified in the text. Intracellular stimulation was delivered at a rate of 0.1 or 0.2 Hz for 10–30 trials per condition. We obtained recordings as described⁴⁵ and filled the pipettes with (in mM) 130 potassium gluconate, 10 sodium gluconate, 10 HEPES, 10 phosphocreatine, 4 Mg-ATP, 2 Na₂-ATP, 0.3 GTP, 4 NaCl and 0.4% biocytin (pH 7.2).

All recordings were done in cortical L5 and L6. We identified L5 by the large size of cell somata. In 70% of our whole-cell recordings of pyramidal cells, where the resting membrane potential was stable and no holding currents were applied, the ongoing AP activity was very low (mean 0.21 ± 0.6 Hz, median 0 Hz). We excluded 6 out of 50 recordings from M1 neurons from analysis because the anaesthesia was considered to be too deep (the criterion was an absence of even, small whisker movements; that is, fewer than one whisker movement of $>0.5^\circ$ per 20 s). Thus, 44 stimulation experiments with 34 regularly spiking and 10 intrinsically bursting cells were analysed.

We made four additional whole-cell recordings to compare anaesthetized and awake conditions. For these, rats (P28–P30; $n = 6$) were prepared for chronic recordings⁴⁵ and habituated to head fixation. On the day of the experiment, rats were anaesthetized with an intraperitoneal dose of a mixture of Dormitor (medetomidine hydrochloride (Roche); 225 μ g per kg), Dormicum (midazolam (Roche); 6 μ g per kg) and fentanyl (Janssen-Cilag) (7.5 μ g per kg). A whole-cell recording was established, and anaesthesia was antagonized with a subcutaneous dose of a mixture of Antisedan (atipamezole (Roche); 1 mg per kg), Anexate (flumazenil (Roche); 600 μ g per kg) and Nalcanti (naloxone (Bristol Myers-Squibb); 180 μ g per kg). Antagonizing anaesthesia required 1–3 min.

Whisker movement analysis

We videotaped whisker movements from directly above, and the angular movements of whiskers of interest were quantified in a frame-by-frame analysis of videotapes. A subset of the data was analysed with specially developed tracking software (Whiskerwatcher, Arrington Research); the precision of the tracking system usually exceeded 0.05° . Whisker position data refer to the ‘best whisker’ (the maximally displaced whisker) determined by extracellular stimulation. We report peak-to-peak poststimulus movement amplitudes (measured as shown in Fig. 3f) rather than the baseline-to-peak amplitudes. Measurement of peak-to-peak poststimulus movement amplitudes and baseline-to-peak amplitudes led to similar results except for 1-AP and 2-AP initiation, where evoked movements often consisted of a small forwards movement followed by a large and slow backwards movement. We also analysed, trial by trial, the root mean square amplitude of whisker movements. This analysis led to quantitative conclusions very similar to the quantification of peak-to-peak amplitudes (data not shown). Movements were sampled at a rate of 25 Hz (in two experiments at both 25 Hz and 150 Hz), which is sufficient to sample the rather slow whisker movements of anaesthetized rats.

Histology

Cells were recovered by histological procedures⁴⁴ and biocytin-labelled neurons were reconstructed with NeuroLucida software (MicroBrightfield). Data are reported as the mean \pm s.e.m. unless otherwise stated.

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