

Activation and measurement of free whisking in the lightly anesthetized rodent

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The rodent vibrissa system is a widely used experimental model of active sensation and motor control. Vibrissa-based touch in rodents involves stereotypic, rhythmic sweeping of the vibrissae as the animal explores its environment. Although pharmacologically induced rhythmic movements have long been used to understand the neural circuitry that underlies a variety of rhythmic behaviors, including locomotion, digestion and ingestion, these techniques have not been available for active sensory movements such as whisking. However, recent work that delineated the location of the central pattern generator for whisking has enabled pharmacological control over this behavior. Here we specify a protocol for the pharmacological induction of rhythmic vibrissa movements that mimic exploratory whisking. The rhythmic vibrissa movements are induced by local injection of a glutamatergic agonist, kainic acid. This protocol produces coordinated rhythmic vibrissa movements that are sustained for several hours in the anesthetized mouse or rat and thus provides unprecedented experimental control in studies related to vibrissa-based neuronal circuitry.

INTRODUCTION

Sensory organs and appendages in animals are typically under active motor control. Perception of objects in the world therefore depends on self-generated movement. It has long been recognized that proper analysis of sensory systems cannot be adequately studied in the absence of such self-generated movement^{1,2}. Different organisms have developed a multitude of strategies for active sensation, including eye and head movements for vision, pinna and head movements for audition, sniffing for olfaction, and arm and finger movements for tactile sensation. Rodents and other mammals are endowed with exquisite arrays of sinus hairs, or vibrissae, that transduce tactile stimuli through mechanoreceptors in their follicles^{3,4}. In rodents, the mystacial pad on the snout contains specialized muscles to control the position of the follicles, each of which contains a vibrissa, in an active sensory behavior known as whisking. The relative simplicity and ease of measurement of whisking has established the rodent vibrissa system as an important model system for the study of active sensation^{5–10}, motor control^{11–15} and sensorimotor integration^{16–18}.

The crucial role of movement in sensation, and vice versa, emphasizes the importance of studying both sensory physiology and motor control in the context of naturally generated active sensory behaviors. Such studies generally require neurophysiological techniques to be performed in alert, behaving and often extensively trained animals^{19–24}. Although these studies are considered the gold standard, they can be exceedingly laborious and are often ill-suited for intracellular recording. An alternative strategy is the use of pharmacological activation of motor patterning circuitry to mimic natural patterns of motoneuron output in intact and semi-intact preparations^{25,26}. Such preparations have yielded valuable data on the neural circuitry that generates rhythmic movements related to locomotion^{27–31}, scratching³² and ingestion³³.

In the rodent vibrissa system, rhythmic stimulation of the facial motor nerve at the whisking frequency has been used to determine the neuronal encoding of tactile stimuli that rodents are likely to encounter during natural whisking^{34–37}. However, unlike pharmacologically induced fictive movement preparations,

electrically induced whisking requires the experimenter to generate the vibrissa kinematics and rhythm arbitrarily on the basis of the stimulation parameters. Facial nerve stimulation typically recruits all of the vibrissa muscles together, with the largest motor fibers recruited first rather than last—the opposite of what would be expected according to Henneman's size principle³⁸—and it further produces high accelerations that are atypical of natural whisking. Although the recent use of use of optogenetic activation may relieve the difficulty with recruitment in the sense that small fibers are recruited first³⁹, it still requires the experimenter to directly control kinematics.

Here we describe a protocol for pharmacological induction of sustained rhythmic vibrissa movements in an *in vivo* anesthetized preparation. Local injection of a glutamatergic agonist, kainic acid, is used to activate pre-motor neuronal networks to mimic natural whisking¹³. This approach is similar to well-established methods that use pharmacological activation to elicit real or fictive rhythmic movements²⁷. In this procedure, sustained rhythmic contractions

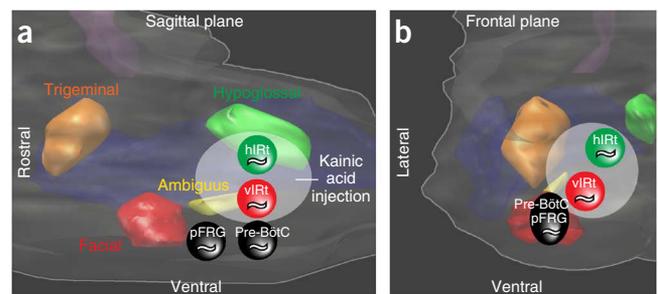


Figure 1 | Target site for local injection of kainic acid to produce rhythmic vibrissa movements. **(a,b)** The pons and medulla contain pools of motoneurons (background) that control the jaw (orange), tongue (green), face (red) and airway (yellow). The intermediate reticular formation (IRF) contains neuronal oscillators for licking (hIRF, green), whisking (vIRF, red) and breathing (black). The target injection site is shown in white in the sagittal **(a)** and **(b)** frontal planes. Breathing is regulated by the pre-Böttinger complex (pre-BötC) and the parafacial respiratory group (pFRG).

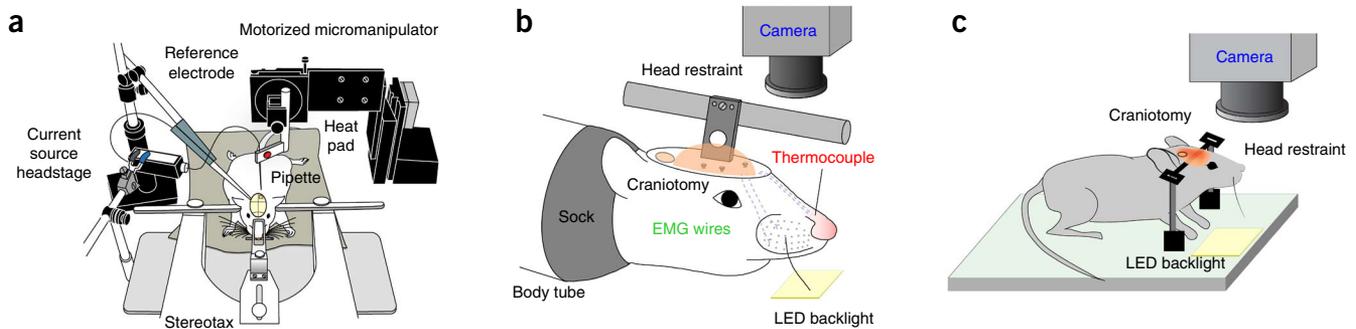


Figure 2 | Diagram of experimental procedures to induce and measure kainic acid–induced vibrissa movements. **(a)** A rat is placed in a stereotaxic holding frame and a craniotomy is made in the bone dorsal to the intermediate reticular formation of the brainstem. Kainic acid is injected through a micropipette, which is lowered into the brainstem via a micromanipulator. **(b)** Following the injection, the rat is implanted with a head-restraining plate and transferred to a jig, which holds the body and head in place. A camera captures the resulting vibrissa movements. Other physiological measures such as EMG recordings from the mystacial pad and breathing measurements through a thermocouple can be monitored simultaneously. The apparatus shown in this panel is for measurements in rats. Adapted from Moore *et al.*¹³, Nature Publishing Group. **(c)** A similar apparatus for mice, except that the head is restrained by a crossbar. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego (UC San Diego).

of the intrinsic vibrissa muscles are induced by focal injection of kainic acid into the medulla. In addition to a detailed protocol to achieve this pharmacologically induced whisking, we provide examples of how this preparation can be used to study sensory physiology, motoneuron physiology and motor control. Finally, we encourage readers to adhere to the ARRIVE^{40,41} and related⁴² guidelines for the reporting of their activation studies.

Experimental design

The protocol described here involves stereotaxic iontophoretic injection of kainic acid in the intermediate reticular formation of the medulla (Fig. 1). After the injection, the animal is implanted with a stainless steel plate for head restraint and the vibrissa motion is monitored in real time (Figs. 2–4). The induction of rhythmic vibrissa movements by kainic acid injection typically lasts for 1–3 h in rats, and 0.5–1 h in mice. Therefore, a variety of scientific questions can be addressed by combining

the protocol with various possible experiments. To demonstrate the scientific utility of the protocol, we describe three specific examples of experiments that we have performed. In the first example, we show that kainic acid–induced whisking produces responses in the vibrissa sensory areas of the brain that report the rhythmic motion, and can therefore be used to study active sensation. In this example, the protocol is combined with stable juxtacellular monitoring of sensory neurons (Fig. 5). In the second example, we demonstrate intracellular recording from motoneurons as they drive movement of the vibrissae. This enables studies of the cellular mechanisms of motoneuron physiology (Fig. 6). In the final example, we show that bilateral injections of kainic acid produce independent motion of the vibrissae on the two sides of the face. These data demonstrate that exploratory whisking, which is normally observed to be bilaterally symmetrical^{24,43}, involves coupling between two potentially independent neuronal oscillators (Fig. 7).

MATERIALS

REAGENTS

- Female mice (C57BL/6; 20–30 g) or rats (Long-Evans; 250–300 g)
- **! CAUTION** Experiments involving rodents must conform all relevant governmental and institutional guidelines and regulations.
- Ketaset/ketamine HCl (Fort Dodge)
- Anased/xylazine solution (Lloyd Laboratories)
- Kainic acid monohydrate (Sigma-Aldrich, cat. no. K0250)
- Tris base (Fisher Scientific, cat. no. BP152-500)
- Dental acrylic (rat: Jet denture repair acrylic, Lang Dental Manufacturing; and mouse: Grip Cement, Dentsply caulk, cat. nos. 675572, 675571)
- Cyanoacrylate glue (mouse: Loctite 401)
- Betadine surgical scrub (Purdue Products)
- PBS (optional; Sigma-Aldrich, cat. no. P3813-10PAK); used for histology
- Biotinylated dextran amine (BDA; optional; MW 10,000 Da; Invitrogen, cat. no. D-1956); used for anatomical tracing
- Streptavidin–Alexa Fluor 488 (optional; Invitrogen, cat. no. S-11223); required to reveal BDA labeling
- Anti-NeuN (optional; Millipore, cat. no. MAB377) and goat anti-mouse Alexa-488 (optional; Invitrogen, cat. no. A21236); used for pan-neuronal somatic labeling
- NeuroTrace fluorescent Nissl stain (optional; Invitrogen, cat. no. N-21483); used for cell somatic labeling

EQUIPMENT

- Quartz capillary tubing (Sutter, cat. no. QF-100-60-10)
- Micropipette puller (Sutter P-2000)
- Rodent stereotaxic frame (Kopf Model 900)
- Stereotaxic mask (rat: Kopf cat. no. 920; and mouse: Kopf, cat. no. 923-B)
- Stereotaxic ear bars (rat: Kopf cat. no. 957; and mouse: Kopf cat. no. 922)
- Servo-controlled heating blanket (rat: Harvard Apparatus, cat. no. 50-7053; and mouse: FHC, cat. no. 40-90-8D)
- Micromanipulator (Sutter MP-285, Sutter, cat. no. ROE200)
- Current source (Molecular Devices, Axoclamp 900A)
- Head-restraint apparatus or bolts (custom, designs available at <http://physics.ucsd.edu/neurophysics/>)
- Bone screws (Small Parts, cat. no. B000FN29YM)
- Body sock (custom design, available at <http://physics.ucsd.edu/neurophysics/>)
- PVC or aluminum tube (~2 3/8-inch inner diameter or equivalent)
- Pipette microloader (Eppendorf, cat. no. 930001007)
- LED backlight (New Haven Display, cat. no. NHD-C128128BZ-FSW-BGW)
- Digital video camera (Basler A602f)
- Data acquisition system with analog input (optional; ADInstruments, Powerlab 8/35); for online monitoring of vibrissa position
- Data acquisition board w/ analog output (optional; National Instruments, cat. no. PCI-6024E); for online monitoring of vibrissa position

PROTOCOL

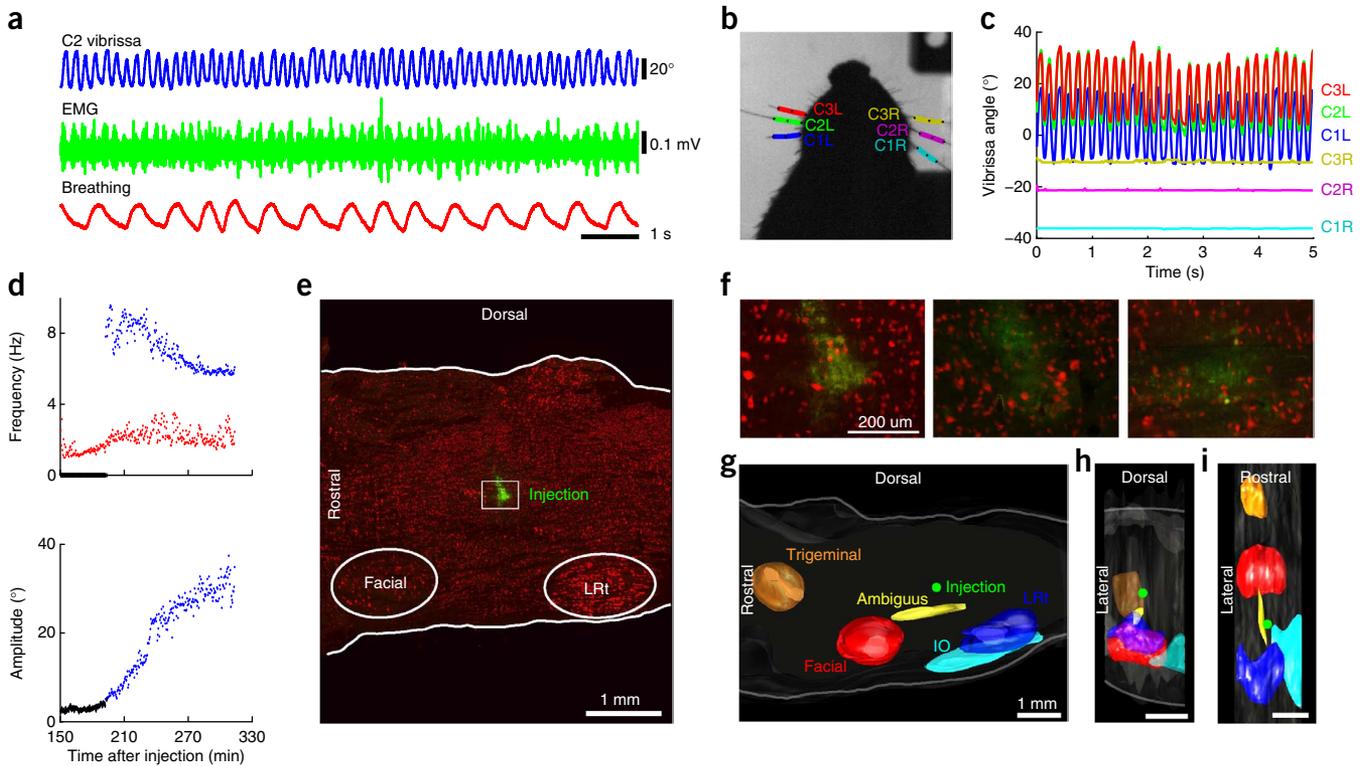


Figure 3 | Kainic acid injection produces rhythmic vibrissa movements in rat. **(a)** Vibrissa position (blue), EMG activity as measured from the mystacial pad (green), and breathing as measured with a thermocouple placed in the nose (red) following kainic acid injection. Similar results for vibrissa position were obtained in a total of 21 out of 27 rats (77%). EMG activity was monitored in four rats, all of which produced similar results. All rats represented in the present and subsequent figures were Long Evans females, 250–350 g, purchased from Charles River Laboratories. **(b)** Six C-row vibrissae in the head-restrained, lightly anesthetized rat were tracked using high-speed videography. **(c)** Angular position of each of the tracked vibrissae relative to the x axis in **b**, 5 h after injection. Videography of multiple vibrissae was recorded in eight rats, all of which produced similar results. **(d)** Time course of vibrissa movement frequency (top, blue) and amplitude (bottom, blue) after kainic acid injection. The frequency is defined as $(1/2\pi) \times d\Phi(t)/dt$ averaged over 30-s intervals, where $\Phi(t)$ represents the instantaneous phase from the Hilbert transform of the vibrissa angle in time³¹. The amplitude is defined as $2 \times A(t)$ averaged over the same interval, where $A(t)$ represents the amplitude from the Hilbert transform. The breathing frequency (top, red) is similarly defined. Frequency was defined only for movements that had an amplitude of $>5^\circ/s$. Vibrissa movements with amplitudes less than this are shown in black. Continuous vibrissa monitoring was performed in 23 rats, 20 of which produced similar results. **(e)** Sagittal section containing the injection site. The injection site was identified as described in Steps 16–20, and counterstained with anti-NeuN. **(f)** Magnified view of the injection site in **e** (white box) is shown in the left panel. Similar injection sites in two other rats in which continuous vibrissa movements were also observed are shown in the middle and right panels. The image in the middle panel was published previously as supplementary information in ref. 13, Nature Publishing Group. NeuN histology was performed on a total of five rats after labeling the injection site with BDA. Labeling was successful in four of the five attempts, and all four cases showed similar NeuN staining around the labeled site. **(g)** Sagittal view of a 3D reconstruction of the injection site **e** relative to anatomical landmarks: trigeminal (orange), facial (red) and ambiguous (yellow) motor nuclei, the inferior olive (IO, light blue), and the lateral reticular nucleus (LRt, dark blue). **(h,i)** Frontal (**h**) and horizontal (**i**) views of the reconstruction in **g**. Reconstructions were performed by scanning all sections on a Nanozoomer Slide Scanner (Hamamatsu) and tracing the anatomical boundaries using Neurolucida software (Microbrightfield). All animal procedures were approved by the IACUC at UC San Diego.

- MATLAB with data acquisition and video acquisition toolboxes, optional (Mathworks); for online monitoring of vibrissa position
- Monitoring software (optional; custom MATLAB code available at <http://physics.ucsd.edu/neurophysics/>); for online monitoring of vibrissa position
- Thermocouple (optional; Omega Engineering, cat. no. 5TC-TT-K-36-36); used for respiration measurements
- Electromyogram (EMG) electrode wires, 0.002-inch insulated tungsten (optional; California Fine Wire Material no. 100211); used for EMG measurements
- Solder and Stay-Clean solder flux (optional; Harris Products); required for EMG implantation
- 27-gauge syringe needle, 1/2-inch (optional; BD 305109); required for EMG implantation
- Amplifier (optional; World Precision Instruments DAM80 or similar); used with EMG implantation

REAGENT SETUP

Kainic acid solution To make the kainic acid solution, first prepare 0.1 M Tris buffer (0.1 M Tris base, adjust pH to 8.3 with HCl). Add 1 ml Tris base

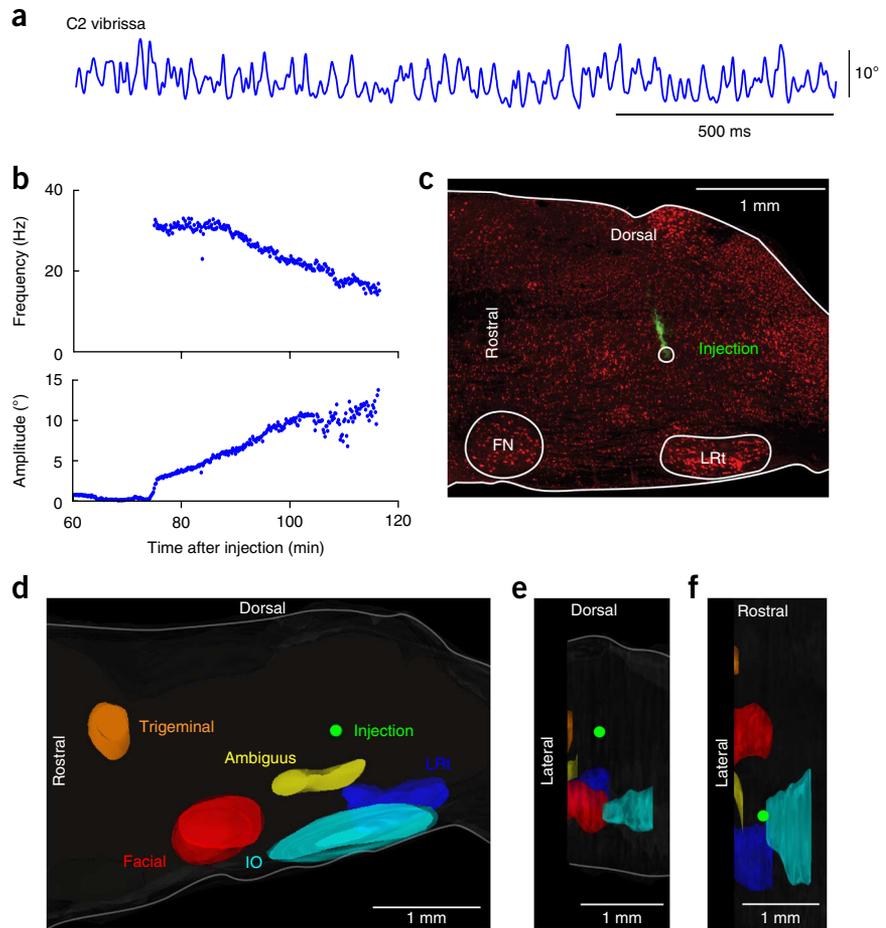
to 10 mg kainic acid powder yielding a 1% (wt/vol) solution. Dispense into 100- μ l aliquots and store them at 4 °C. We have successfully stored the solution at 4 °C for months. If you wish to label the injection site, add BDA (2% (wt/vol)) immediately before use.

Artificial cerebral spinal fluid (ACSF) Combine 125 mM NaCl, 10 mM glucose, 10 mM HEPES, 3.1 mM CaCl_2 and 1.3 mM MgCl_2 and adjust the mixture to pH 7.4. Store ACSF at 4 °C, but freshly prepare new batches on a weekly basis.

EQUIPMENT SETUP

Fig Before the experiment, construct a jig for monitoring the lightly anesthetized rodent. To do this, secure the body-restraint tube and the head-restraint apparatus in the appropriate configuration (**Fig. 2**). A variety of materials can be used, but we recommend using standard optomechanical bench parts. Mount the camera so that the head-restraint apparatus is in the field of view. Install the necessary computer hardware and software to monitor the vibrissae and any other data of interest. Online monitoring of vibrissa position (**Figs. 3–6**) can be achieved with a data acquisition card, software drivers for the camera and the custom MATLAB vibrissa monitoring software listed in the Equipment section.

Figure 4 | Kainic acid injection produces rhythmic vibrissa movements in mouse. (a) Vibrissa position (blue) following kainic acid injection. Similar results for vibrissa position were obtained in a total of 7 out of 12 mice (58%). All mice were C57BL/6 females, 20–30 g, purchased from Jackson Laboratories. (b) Time course of vibrissa movement frequency (top) and amplitude (bottom) after kainic acid injection. Conventions are as in **Figure 3d**. (c) Sagittal section containing the injection site, prepared as in **Figure 3e**. NeuN histology was performed on a total of five mice after labeling the injection site with BDA. Labeling was successful in five of the five attempts, and all five cases showed similar NeuN staining around the labeled site. (d–f) 3D reconstruction of the injection site in **c** relative to anatomical landmarks; conventions are as in **Figure 3g–i**, respectively. All animal procedures were approved by the IACUC at UC San Diego.



Injection pipettes To prepare the injection pipettes, first solder bare silver wire (0.01-inch diameter) to an appropriate connector pin for the current source. Pull pipettes on the pipette puller from quartz capillary tubing so that tip diameters are 1 μm or less. Then break or grind the tips to an inner diameter of 10–15 μm for rat injections or an inner diameter of 3–5 μm for mouse injections. Immediately before the experiment, fill the pipette with ~5 μl of kainic acid solution, ensuring that there are no air bubbles in the tip (see TROUBLESHOOTING table). We recommend using an Eppendorf microloader for this.

EMG recording electrodes If EMG recordings are to be performed, prepare the electrodes before the experiment. First, cut two strips of ~6 cm of 0.002-inch Teflon-coated tungsten wire. By using fine-tip forceps, strip the Teflon ~0.5–1 mm from the front end, and ~1 cm from the back end. Insert the back end into a connector pin appropriate for the extracellular amplifier and solder the wire in place. Soldering tungsten wire requires solder

flux. Next, remove the plastic connector from a 27-gauge needle. This can be achieved by first heating the plastic with a hot air gun, and then pulling the needle from its housing. Insert the front end of one wire into the blunt end of the needle and push it through so that it is visible from the sharp end of the needle. Bend the front end of the wire backwards at the tip to keep it from slipping during insertion. Do the same for the second wire.

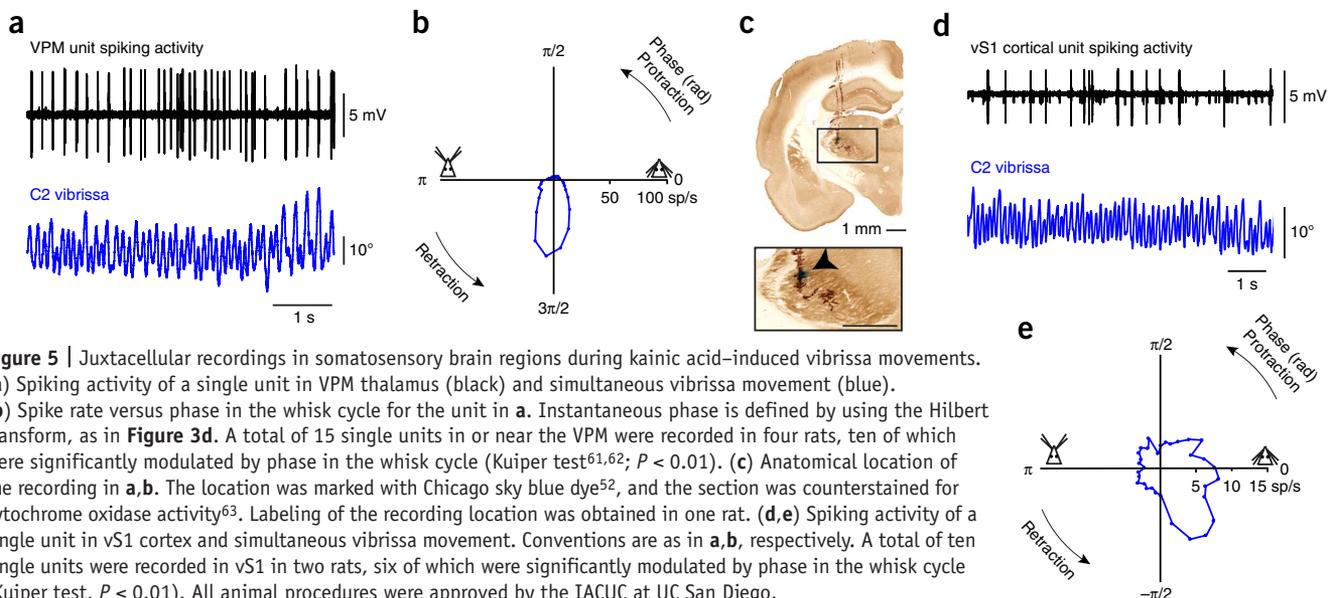


Figure 5 | Juxtacellular recordings in somatosensory brain regions during kainic acid-induced vibrissa movements. (a) Spiking activity of a single unit in VPM thalamus (black) and simultaneous vibrissa movement (blue). (b) Spike rate versus phase in the whisk cycle for the unit in **a**. Instantaneous phase is defined by using the Hilbert transform, as in **Figure 3d**. A total of 15 single units in or near the VPM were recorded in four rats, ten of which were significantly modulated by phase in the whisk cycle (Kuiper test^{61,62}; $P < 0.01$). (c) Anatomical location of the recording in **a, b**. The location was marked with Chicago sky blue dye⁵², and the section was counterstained for cytochrome oxidase activity⁶³. Labeling of the recording location was obtained in one rat. (d, e) Spiking activity of a single unit in vS1 cortex and simultaneous vibrissa movement. Conventions are as in **a, b**, respectively. A total of ten single units were recorded in vS1 in two rats, six of which were significantly modulated by phase in the whisk cycle (Kuiper test, $P < 0.01$). All animal procedures were approved by the IACUC at UC San Diego.

PROTOCOL

Figure 6 | Intracellular recording in a facial motoneuron during kainic acid-induced vibrissa movements. (a) Schematic of the recording setup. Conventions are as in **Figure 1a**. (b) Example recording with membrane potential shown in black and vibrissa motion shown in blue. Similar recordings were obtained in a total of nine cells in four rats. All animal procedures were approved by the IACUC at Laval University.

PROCEDURE

Surgery

1| Follow option A for rats and option B for mice.

(A) Rat surgery

- (i) Administer ketamine/xylazine anesthesia via i.p. injection. Inject a cocktail that contains 90 mg/kg (reagent-to-animal weight) ketamine and 5 mg/kg xylazine. Wait ~5–10 min for the anesthesia to take effect, and then check the withdrawal reflex by pinching the toe of the animal. Proceed once the reflex is lost.
 - (ii) Shave the fur on top of the cranium. Disinfect the exposed skin with Betadine. Then place the animal in a stereotaxic holding frame, attach the servo-controlled heating blanket and turn it on to 37 degrees.
 - (iii) Use a scalpel to make an incision in the skin above the cranium, approximately along the midline. Scrape away the periosteum with a spatula.
 - (iv) Estimate the approximate stereotaxic coordinates of the injection site with a ruler and mark the site and the location of bregma with a permanent pen. For 250–300-g Long-Evans female rats, the appropriate injection site is 12.7 mm caudal and 1.5 mm lateral to the bregma suture.
 - (v) Use a dental or electric drill to perform a craniotomy, a 2–3 mm square, that is centered at the coordinates in Step 1A(iv). Carefully remove the dura mater using fine forceps. Bathe the open cranium with ACSF.
 - (vi) Place the pipette filled with the kainic acid solution in the jig that attaches the pipette to the micromanipulator. To label the recording site (optional), the pipette should contain a mixture of kainic acid and BDA. Connect the pipette to the current source via the silver wire. Place the reference/return electrode so that it touches the cranial surface. By using the micromanipulator, move the pipette to the coordinates listed in Step 1A(iv). Lower the pipette until it touches the pial surface. Then slowly lower the pipette into the brain to a depth of 7.4 mm below the pial surface.
 - (vii) Use the current source to pass negative current pulses. Pulses should have an amplitude of -500 nA and be 250 ms in duration at a repetition period of 500 ms for 10 min. If the recording site is being labeled, pass additional pulses of $+700$ nA for 10 min to electropore the BDA. In all cases, monitor the applied current or voltage at the current source.
- ### ? TROUBLESHOOTING
- (viii) Leave the pipette in the brain for 5 min with no current flow. Then slowly raise and remove the pipette with the micromanipulator.

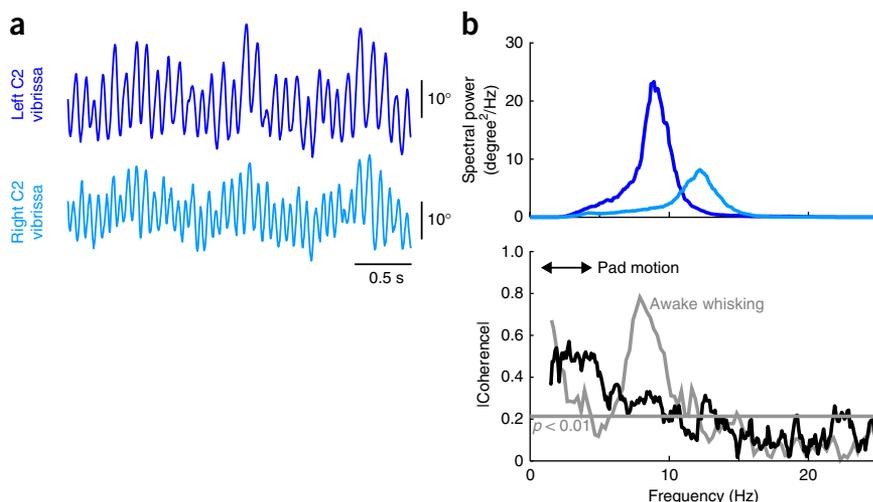
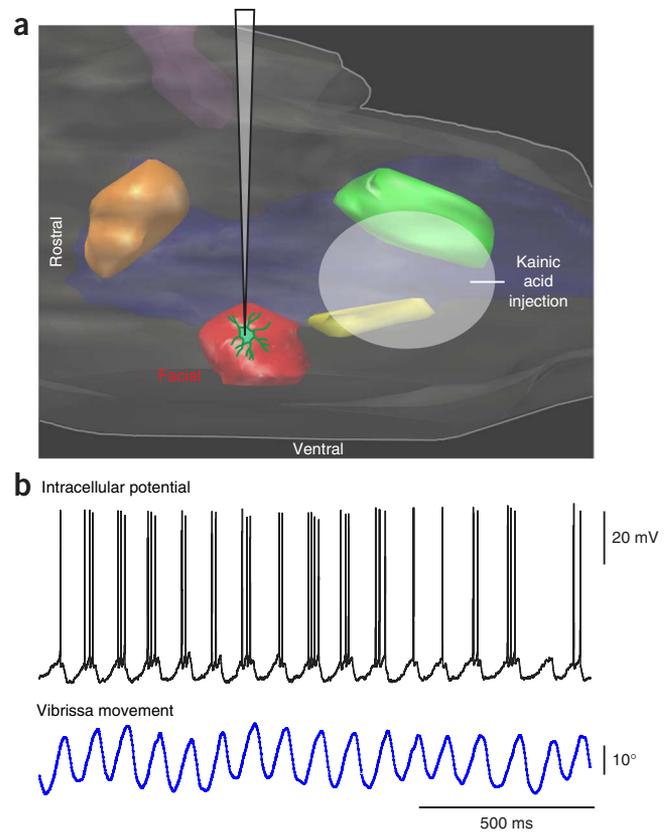


Figure 7 | Bilateral kainic acid injection produces independent vibrissa movements on the left and right sides of the face. (a) Movement of the left (dark blue) and right (light blue) C2 vibrissae after kainic acid injections. Similar results were obtained in a total of two rats. (b) Power spectra (top; dark and light blue) and spectral coherence (bottom; black) between the movements of each of the vibrissae in a. The two signals show low coherence in the band of whisking frequencies relative to control data for bilateral active whisking in alert animals (bottom; gray). Control data are from Fee *et al.*²⁴. All animal procedures were approved by the IACUC at UC San Diego.

- (ix) Drill holes into the cranium that are the diameter of the #0–80 screws, and insert the screws. Screws should be placed in each of the cranial plates. Screw in the screws no more than two and a half turns to ensure that the screws do not penetrate the brain. If additional surgery is required for the desired experiment, it can be performed at this point. In **Figure 5**, for example, an additional craniotomy is performed over ventral posterior medial (VPM) thalamus or primary vibrissa sensory (vS1) cortex. Alternative rodent head-restraint protocols have been described previously⁴⁴, and can be used in place of Step 1A(ix–xi).
- (x) By using the micromanipulator, lower the head-restraint piece to its proper location on the cranium.
- (xi) Apply dental acrylic to the base of the head-restraint piece and the heads of each of the bone screws. The dental acrylic should form one contiguous mass to secure the screws to the implant. Wait at least 10 min for the cement to dry, and check that it is solid before continuing. If the desired experiment requires access to the brain, be sure to leave any additional craniotomies uncovered. If stereotaxic measurements are also required, make sure that you leave the necessary cranial landmark(s), e.g., the bregma and lambda sutures, free of cement.
- (xii) Remove the animal from the stereotaxic holding frame. Place the animal inside the body sock. Tighten the rostral drawstring of the sock securely around the sternum and the caudal drawstring around the tail. The use of the body sock is not strictly necessary. However, under light ketamine/xylazine anesthesia, several hours after induction, the rat may occasionally move the forepaws or hindpaws. This can obstruct imaging of the vibrissae and, in extreme cases, may produce torque on the head restraint. The use of the sock is recommended to restrict these movements and thus ensure the safety of the animal.

(B) Mouse surgery

- (i) Administer ketamine/xylazine anesthesia via i.p. injection. Inject a cocktail that contains 100 mg/kg ketamine (reagent-to-animal weight) and 10 mg/kg xylazine. Wait ~5–10 min for the anesthesia to take effect, and then check the withdrawal reflex by pinching the toe of the animal. Proceed once the reflex is lost.
- (ii) Shave the fur on top of the cranium. Disinfect the exposed skin with Betadine. Then place the animal in a stereotaxic holding frame, attach the servo-controlled heating blanket and turn it on to 37 degrees.
- (iii) Use a scalpel to make an incision in the skin above the cranium, approximately along the midline. Scrape away the periosteum with a spatula.
- (iv) Estimate the approximate stereotaxic coordinates of the injection site with a ruler and mark the site and the location of bregma with a permanent pen. For 25 g mice, the coordinates are 6.5 mm caudal and 0.9 mm lateral to the bregma suture.
- (v) Score the cranium in a cross-hatch pattern with a scalpel. Leave space around the area where the craniotomy will be performed for the kainic acid injection (performed in Step 1B(vi)). Glue the sutures with cyanoacrylate to provide stability during drilling. Any additional surgery and positioning of the head restraint required for the desired experiment can be performed at this point, before kainic acid injection, to maximize recording time after kainic acid injection.
- (vi) By using the micromanipulator, lower the head-restraint piece to its proper location on the cranium. Coat the scored areas of the cranium and glue the head-restraint piece with cyanoacrylate. Wait at least 20 min for the cyanoacrylate to dry completely.
- (vii) Use a dental or electric drill to perform a craniotomy, a 2–3-mm square that is centered at the coordinates in Step 1B(iv). Carefully remove the dura mater by using fine forceps. Bathe the open cranium with ACSF.
- (viii) Place the pipette filled with the kainic acid solution in the jig that attaches the pipette to the micromanipulator. To label the recording site (optional), the pipette should contain a mixture of kainic acid and BDA. Connect the pipette to the current source via the silver wire. Place the reference/return electrode so that it touches the cranial surface. Use the micromanipulator to move the pipette to the coordinates listed in Step 1B(iv). Lower the pipette until it touches the pial surface. Then slowly lower the pipette into the brain to the appropriate depth of 4.5 mm.
- (ix) Use the current source to pass negative current pulses. Pulses should have an amplitude of –350 nA and be 1 s in duration at a repetition period of 2 s for 5 min. If the recording site is being labeled, pass additional pulses of +300 nA for 10 min to electrophorese the BDA. In all cases, monitor the applied current or voltage at the current source.

? TROUBLESHOOTING

- (x) Leave the pipette in the brain for 1 min with no current flow. Then slowly raise and remove the pipette with the micromanipulator.
- (xi) Apply dental cement to the exposed areas of the cranium and over the head-restraint piece. If the desired experiment requires access to the brain, be sure to leave any additional craniotomies uncovered. If stereotaxic measurements are also required, make sure that you leave the necessary cranial landmark(s), e.g., the bregma and lambda sutures, free of cement.
- (xii) Remove the animal from the stereotaxic holding frame.

Vibrissa monitoring

2| Place the animal in the body-restraint tube and attach the head-restraint piece to the holding jig. For mice, keep the animal on a heating pad after placing it in the holding jig. If EMG electrodes are used to monitor muscle activity (**Fig. 3a**), insert them into the mystacial pad and attach them to the amplifier at this point. The wires can be inserted by pushing the 27-gauge needle into the mystacial pad using a hemostat and then retracting the needle. The needle should be inserted dorsally to the A1 vibrissa and pushed ventrally, rostrally and laterally toward the center of the pad. The needle should be pushed in until its tip produces a bulge near the C row vibrissae before it is retracted. The needle should remain around the EMG wire leads but not touch the animal or obstruct the camera view of the vibrissae.

? TROUBLESHOOTING

3| Open the vibrissa-monitoring software and adjust the camera position so that the vibrissa(e) and backlight are in view. Set the software parameters. If the suggested line-scan hardware and software are being used, trim the animal to a single vibrissa. Details regarding the use of this software are included with the download. Begin vibrissa monitoring, along with monitoring of any other physiological signals of interest. In **Figure 3**, we monitor mystacial pad EMG and breathing.

? TROUBLESHOOTING

Optional experimental procedures

4| Perform any additional procedures required for the desired experiment at this point. In the example experiment shown in **Figure 5**, we monitored neuronal activity in the thalamus or cortex along with the vibrissa movement. In the example experiment shown in **Figure 6**, we monitored facial motoneurons intracellularly and, in the example experiment shown in **Figure 7**, we made sequential kainic acid injections in both hemispheres.

5| If the injection site was labeled, or if other neuroanatomical methods requiring a survival time longer than 6 h are desired, cover the craniotomy with ACSF-soaked surgical foam. Suture the initial midline incision up to the dental acrylic that holds the head-restraint plate. Cover the skin around the plate with additional dental acrylic so that the surgical site is closed. Alternatively, proceed directly to the next step.

Optional histological procedures

6| At the chosen experimental endpoint, transcardially perfuse the animal and extract the brain. If labeling the injection site with BDA, the animal should not be perfused until at least 48 h after injection to assess whether any neuronal damage has occurred.

7| If desired, section the brain at 30 μm on a freezing microtome and collect sections in well-plates containing PBS. Rinse the sections two times with PBS.

8| If you wish to visualize BDA that was used as a tracer, add the solution containing streptavidin–Alexa Fluor 488 and incubate the sections for 90–120 min. Rinse the sections twice with PBS.

9| Perform desired immunohistochemical procedures, and mount sections on slides according to standard practice. The use of anti-NeuN or Neurotrace fluorescent Nissl stain is recommended as a means to determine cytological and nuclear boundaries.

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible reason	Solution
Equipment setup	Air bubbles in the pipette tip	Solution does not wick properly	Hold the pipette between your thumb and forefinger and gently tap or flick it with the other forefinger. Alternatively, while holding the pipette, strike your hand on a hard surface (table or lab bench), ensuring that the pipette does not directly contact the surface

(continued)



TABLE 1 | Troubleshooting table (continued).

Step	Problem	Possible reason	Solution
1A(vii) or 1B(ix)	Applied current does not reach the level set, or the voltage on the current source reaches its compliance limit	Pipette is clogged, or the compliance of the current source is too low	Slowly remove the pipette and repeat the injection with a new one. Alternatively, use a higher compliance source; we recommend a compliance of 100 V
	Animal breathing becomes irregular, or the animal dies	Too much kainic acid is being injected, or the site is too close to crucial respiratory centers	There are several possible solutions: first, stop injecting current and wait for 5 min while monitoring the animal's breathing. If breathing recovers to its regular rhythm, try moving the pipette dorsally 200–400 μm and continuing the injection, and/or lowering the current amplitude
2	EMG electrodes do not stay in place in the muscle	Crease in the wires is not strong enough, or wire is not inserted into muscle	Re-fold the wires ~0.5–1 mm from the tips. Insert the needle as described in Step 2, until its tip produces a bulge near the C-row of the mystacial pad. Apply pressure to the bulge in the pad with your forefinger while retracting the needle. This should hold the wires in place as the needle is being removed. Be sure that the needle does not exit the rat's skin while you are applying pressure
3	Vibrissa goes out of the focal plane of the camera and is blurry or not detected by the software	Movement of the animal or change in muscle tension	Stop the vibrissa monitoring software, re-focus the camera, and restart it
	Animal begins to move	Anesthesia is wearing off	If sufficient data have been collected, it may be advisable to stop the experiment at this point. It is possible to re-inject a supplemental dose of ketamine (one-third of the initial dose); however, this may cause the amplitude of vibrissa movement to decrease

● TIMING

Equipment for holding head-restrained, lightly anesthetized rodents should be prepared at least 1 d before beginning the experiment, along with installation of the camera and software for monitoring the vibrissae, as well as preparation of all solutions. On the day of the experiment, the animal is first prepared for the injection by performing a craniotomy over the medulla, which takes ~40 min (Step 1A(i–v) for rats; and Step 1B(i–vii) for mice). The injection itself takes 30–40 min (Step 1A(vi–viii) for rats; and option Step 1B(viii–x) for mice). At this point, if the injection is successful, the animal can be prepared for head restraint (Step 1A(ix–xi) for rats; and Step 1B(xi–xii) for mice) and attached to the head-restraining apparatus (Step 2), which takes another 40 min. Initializing the digital videography software (Step 3) takes 5–10 min. Preparation for additional measurements (Figs. 3 and 5–7) can also be performed at this time (Steps 2 and 4; time varies depending on the experiment). Once the animal is situated on the head-restraint apparatus there is a variable waiting period to observe movement. In rats, small continuous rhythmic movements of all vibrissae can be observed 1–3 h after the injection, and larger-amplitude (10°–30° peak to trough) movements after 2–4 h. The movement continues for several hours even when the animal becomes alert after the anesthesia wears off. After the experiment, the animal can be killed or the surgical site can be closed (Step 5, 15 min) and the animal can be placed in its home cage to recover. If the recording site is labeled (Figs. 3 and 4), or other *post hoc* histological procedures are to be performed as part of the experiment (Fig. 5), the animal can be transcardially perfused (Step 6, 30 min) up to 72 h after the experiment. Optional *post hoc* histological procedures can be performed on the following day (Steps 7–9). The timing of these steps is variable depending on the histological procedures to be performed.

ANTICIPATED RESULTS

Vibrissa movements are initially small, i.e., <2°, after the injection of kainic acid, and they may only be observed on one or several vibrissae. In rats, continuous larger-amplitude rhythmic movement of 10°–30° can be observed after 2–4 h. In our



hands, sustained vibrissa movements are obtained in ~75% of injection attempts (see TROUBLESHOOTING table). The movement is the result of rhythmic contraction of the intrinsic vibrissa muscles at a frequency that is incommensurate with breathing, which remains at a basal level (**Fig. 3a**). The vibrissae ipsilateral to the injection site move in synchrony, whereas the contralateral vibrissae remain stationary (**Fig. 3b,c**). The whisking amplitude and frequency as well as the breathing frequency are shown in **Figure 3d**. The amplitude increases over time and as the frequency decreases, and the continuous oscillation continues for several hours even after the effects of anesthesia wear off and the animal is alert (not shown). The injection site for the experiment in **Figure 3a–d** is shown in **Figure 3e,f**. A 3D reconstruction of the injection site in **Figure 3e** reveals that it is located in the intermediate reticular formation, dorsal to the pre-Bötzing complex (**Fig. 3g–i**). With this injection protocol we do not observe excitotoxic lesions in the medullary reticular formation, as evidenced by staining for neuronal nuclear protein (NeuN) 3 d after the injection^{45,46} (**Fig. 3e,f**). Similar results are observed in mice (**Fig. 4**).

The vibrissa movements shown in **Figures 3** and **4** appear qualitatively similar to those observed during self-generated whisking in alert, behaving rats^{12,47–49}. As such, we propose that this preparation can be an effective means to study active sensation or motor control by combining it with other measurements that are difficult or impossible in alert animals. As noted previously¹³, there can be a substantial delay between the kainic-acid injection and stable, coordinated vibrissa movement (**Fig. 3d**). This time frame allows substantial time to prepare these additional measurements. Several examples of experiments that can be done in conjunction with this preparation are described below.

Example experiment 1: sensory re-afference

We looked at whether neurons in somatosensory areas of the brain respond to self-generated motion in the kainic acid model of whisking, as has been shown previously in the trigeminal ganglion, VPM thalamus and vS1 cortex of freely whisking rats and mice^{19,22–24,50,51}. To make these measurements, we performed a second craniotomy over VPM thalamus or vS1 cortex following the injection of kainic acid (Step 1A(ix) for rat). We then proceeded with Steps 1A(x–xii), 2 and 3. For Step 4, we lowered a pipette electrode into the thalamus or cortex using the micromanipulator. **Figure 5a** shows a juxtosomal recording of a neuronal unit in VPM thalamus along with kainic acid–induced whisking in a rat. This unit spikes preferentially in phase with retraction, as the vibrissa is moving in the caudal direction (**Fig. 5b**). After the recording, the location of the electrode was labeled by iontophoretic injection of Chicago sky blue dye⁵², and was located in the macrovibrissae region of VPM thalamus (**Fig. 5c**). An analogous recording in vS1 cortex showed a unit tuned to the protraction phase, as the vibrissa was moving rostrally (**Fig. 5d,e**).

Example experiment 2: motoneuron physiology

Although it is known that whisking is controlled by motoneurons in the lateral division of the facial motor nucleus⁵³, the inputs to these motoneurons remain uncharacterized. The kainic acid preparation allows us to stably record intracellularly from motoneurons using sharp pipette electrodes. By following the steps of the previous example, we lowered a pipette electrode into the brainstem in a head-restrained lightly anesthetized rat. The data in **Figure 6** show the membrane potential of a facial motoneuron along with kainic acid–induced vibrissa movements. Such recordings enable the temporal relationship between motoneuron postsynaptic potentials and vibrissa movement to be determined.

Example experiment 3: bilateral coordination of whisking

Natural whisking is normally bilaterally coordinated^{11,43}, at least in the absence of external objects⁵⁴ or head turning⁵⁵. In this example, we investigated whether such coordination is obligate, or whether the animal can, in principle, control the two sides independently. Because kainic acid–induced whisking selectively induces whisking on the ipsilateral side, we use a double injection of kainic acid to determine whether the resulting motion on the two sides is phase-locked. For this experiment, two craniotomies were performed in Step 1A(v). Immediately after the injection on one side (Step 1A(viii)), a second injection was administered on the contralateral side (Step 1A(vi–viii)), and the motion of corresponding vibrissae on either side of the face was monitored simultaneously (**Fig. 7a**). The oscillations of the two vibrissae occurred at slightly different frequencies (**Fig. 7b**, top) and had low coherence at either of the whisking frequencies (**Fig. 7b**, bottom). This experiment demonstrated that the left and right sets of vibrissae are controlled by unilateral neuronal oscillators that are phase-locked but that can, in principle, decouple.

Additional potential applications

Local injection of kainic acid produces sustained, coordinated, rhythmic movements of the ipsilateral vibrissae. The frequency and dynamics of these movements are similar to those observed during natural exploratory whisking and, as such, we propose that they can be used to mimic natural exploratory movements. One potential caveat is that, although the amplitude and frequency of whisking change in a predictable manner over the course of the experiment, the whisking pattern is not readily modifiable by the experimenter once it is induced. Thus, electrical stimulation of the nerve^{35,36} may be preferable,



if starting and stopping or rapidly changing the kinematics of whisking is required. Nonetheless, the vibrissa movements described here can be induced in a controlled manner that permits systematic *in vivo* physiological measurements that are difficult or impossible in behaving animals.

In addition to the applications shown in this protocol (Figs. 3–7), the kainic acid procedure is likely to be particularly well suited to study related aspects of motor control. This protocol could be used to determine the effects of different neuromodulators on the dynamics of rhythmic vibrissa movements. For example, the procedure could be used in conjunction with systemic or focal injections of neurotransmitter agonists and antagonists to determine their effects on the frequency, amplitude or offset of ongoing rhythmic vibrissa movements^{56–59}. In addition, as this procedure produces a reliable, easily measurable and easily quantifiable motor output, it could be used to study the effects of surgical or other interventions on peripheral nerve regeneration after acute injury^{44,60}.

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