HHS Public Access

Author manuscript

Nat Protoc. Author manuscript; available in PMC 2016 July 06.

Published in final edited form as:

Nat Protoc. 2014 August ; 9(8): 1792–1802. doi:10.1038/nprot.2014.119.

Activation and measurement of free whisking in the lightly anesthetized rodent

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Abstract

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. While pharmacologically-induced rhythmic movements have been long utilized 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 like 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 the rhythmic vibrissa movements that mimic exploratory whisking. 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

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AUTHOR CONTRIBUTIONS

M.D., D.K., and J.D.M. planned the experiments. M.D., A.K., and J.D.M. performed the experiments. A.K. and J.D.M. analyzed the data. D.K. and J.D.M. wrote the paper. D.K. dealt with the myriad of university organizations that govern animal health and welfare, surgical procedures, and laboratory health and safety issues that include specific oversight of chemicals, controlled substances, human cell lines, lasers, and viruses.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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 critical role of movement in sensation, and vice versa, belays the importance to study both sensory physiology in 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]. While these studies are considered the gold standard, they can be exceedingly laborious and are often ill-suited for intracellular recording. An alternative strategy is use 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], scratching[32], and ingestion[33].

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 based on 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 to that expected from Henneman's size principle[38], and further produces high accelerations that are atypical of natural whisking. The use of optogenetic activation may relieve the difficulty with recruitment in the sense that smallest fibers are recruited first[39].

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 premotor neuronal networks to mimic natural whisking[13]. This approach is similar to well established methods that use pharamocolgical activation to elicit real or fictive rhythmic movements[27]. In this procedure, sustained rhythmic contractions 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. Lastly, we encourage readers to adhere to the [40, 41] and related[42] guidelines for the reporting of their activation studies.

EXPERIMENTAL DESIGN

The protocol described here involves stereotaxic iontophoric 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 to 4). The induction of rhythmic vibrissa movements by kainic acid injection typically lasts for one to three hours in rats, and one-half to one hour 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 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 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

- Mice or rats; experiments involving rodents must conform to national and institutional guidelines and regulations.
- Ketaset/Ketamine HCl (Fort Dodge)
- Anased/xylazine solution (Lloyd Laboratories)
- Kainic acid monohydrate (Sigma K0250)
- TRIS base (Fisher Scientific BP152-500)
  - Dental acrylic (Rat: Jet denture repair acrylic, Lang Dental Manufacturing; Mouse: Grip Cement, Dentsply Caulk 675572, 675571)
  - Cyanoacrylate (Mouse: Loctite 401)
  - Betadine surgical scrub (Purdue Products)
  - Optional: Phosphate buffered saline (Sigma P3813-10PAK); used for histology
- Optional: biotinylated dextran amine (BDA) MW 10000 (Invitrogen D-1956); used for anatomical tracing
- Optional: streptavidin Alexa-488 (Invitrogen S-11223); required to reveal BDA labeling.
- Optional: anti-NeuN (Millipore MAB377) and Goat anti-mouse Alexa-488 (Invitrogen A21236); used for pan neuronal somatic labeling.
- Optional: NeuroTrace Fluorescent Nissl stain (Invitrogen N-21483); used for cell somatic labeling.

Equipment

- Quartz capillary tubing (Sutter QF-100-60-10)
- Micropipette puller (Sutter P-2000)
- Rodent stereotaxic (Kopf Model 900)
- Stereotaxic mask (Rat: Kopf 920; Mouse: 923-B)
- Stereotaxic ear bars (Rat: Kopf 957; Mouse: Kopf 922)
- Servo-controlled heating blanket (Rat: Harvard Apparatus 50-7053; Mouse: FHC 40-90-8D)
- Micromanipulator (Sutter MP-285, ROE200)
- Current source (Molecular Devices, Axoclamp 900A)
- Head-restraint apparatus or bolts (custom, designs available on physics.ucsd.edu/neurophysics/)
- Bone screws (Small Parts B000FN29YM)
- Body sock (custom design, available on physics.ucsd.edu/neurophysics/)
- PVC or aluminum tube (approximately 2-3/8″ inner diameter or equivalent)
- Pipette microloader (Eppendorf 930001007)
- LED backlight (New Haven Display NHD-C128128BZ-FSW-BGW)
- Digital video camera (Basler A602f)
- Optional: Data acquisition system with analog input (ADInstruments Powerlab 8/35); for online monitoring of vibrissa position
- Optional: Data acquisition board w/analog output (National Instruments PCI-6024E); for online monitoring of vibrissa position
- Optional: MATLAB with data acquisition and video acquisition toolboxes (Mathworks); for online monitoring of vibrissa position
- Optional: Monitoring software (custom MATLAB code available on physics.ucsd.edu/neurophysics/); for online monitoring of vibrissa position
- Optional: thermocouple (Omega Engineering 5TC-TT-K-36-36); used for respiration measurements.
- Optional: electromyogram (EMG) electrode wires 0.002″ insulated tungsten (California Fine Wire Co. Material No 100211); used for EMG measurements.
- Optional: solder and Stay-Clean solder flux (Harris Products); required for EMG implantation.
- Optional: 27-guage syringe needle, 1/2″ (BD 305109); required for EMG implantation.

- Optional: Amplifier (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 % (w/v) solution. Aliquot into 100 μL aliquots and store at 4 °C. If you wish to label the injection site, add BDA (2 % (w/v)) 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 to pH 7.4. Store at 4°C but make fresh on a weekly basis.

Equipment Setup

Jig—Before the experiment, construct a jig for monitoring 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 **Equipment**.

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

EMG recording electrodes—If EMG recordings are to be performed, prepare the electrodes prior to the experiment. First cut two strips of approximately 6 cm of 0.002″ Teflon-coated tungsten wire. Using fine-tip forceps, strip the Teflon approximately 0.5 to 1 mm from the front end, and approximately 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 90° at approximately 1 mm from the tip. Do the same for the second wire.

PROCEDURE

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

A. Rat surgery

i. Administer ketamine/xylazine anesthesia via intraperitoneal injection. Inject a cocktail that contains 90 mg/kg (reagent-to-animal weight) ketamine and 5 mg/kg xylazine. Wait approximately 5 to 10 minutes 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 to 300 gram 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 make a craniotomy, a 2 to 3 mm square, that is centered at the coordinates in step iv. Carefully remove the dura mater using fine forceps. Bathe the open craniotomy with artificial cerebrospinal fluid.

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. Using the micromanipulator, move the pipette to the coordinates listed in Step 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 minutes. If the recording site is being labeled, pass additional pulses of +700 nA for 10 minutes. In all cases, monitor the applied current or voltage at the current source (see **TROUBLESHOOTING**).

viii. Leave the pipette in the brain for 5 minutes with no current flow. Then slowly raise and remove the pipet with the micromanipulator.

ix. Drill holes in 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 2-½ turns to ensure the screws do not penetrate the brain.

Optional: If additional surgery is required for the desired experiment, it can be done at this point. In Fig. 5, for example, an additional craniotomy is made over ventral posterior medial (VPM) thalamus or primary vibrissa sensory (vS1) cortex.

x. 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 minutes 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 to leave the necessary cranial landmark(s), *e.g.*, the bregma and lambda sutures, free of cement.

Optional: Alternative rodent head-restraint protocols have been described previously[44]*, and can be used in place of Steps ix to xi.*

xii. Remove the animal from the stereotaxic holding frame. Place the animal inside the body sock. Tighten the rostral drawstring securely around the sternum and the caudal drawstring around the tail.

Optional: 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 intraperitoneal injection. Inject a cocktail that contains 100 mg/kg ketamine (reagent-to-animal weight) and 10 mg/kg xylazine. Wait approximately 5 to 10 minutes 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 gram mice, the coordinates are 6.5 mm caudal and 0.9 mm lateral to the bregma suture.

v. Score the skull 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 vi). Glue the sutures with cyanoacrylate to provide stability during drilling. If additional surgery is required for the desired experiment, it can be done at this point.

Optional: Any additional surgery and positioning of the head restraint can be done at this point, prior to kainic acid injection, to maximize recording time after kainic acid injection.

vi. Using the micromanipulator, lower the head-restraint piece to its proper location on the cranium. Coat the scored areas of the skull and glue the head-restraint piece with cyanoacrylate. Wait at least 20 minutes for the cyanoacrylate to dry completely.

vii. Use a dental or electric drill to make a craniotomy, a 2 to 3 mm square, that is centered at the coordinates in step iv. Carefully remove the dura mater using fine forceps. Bathe the open craniotomy with artificial cerebrospinal fluid.

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. Using the micromanipulator, move

the pipette to the coordinates listed in Step 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 minutes. If labelling the recording site, pass additional pulses of +300 nA for 10 minutes. In all cases, monitor the applied current or voltage at the current source (see **TROUBLESHOOTING**).

x. Leave the pipette in the brain for 1 minute with no current flow. Then slowly raise and remove the pipette with the micromanipulator.

xi. Apply dental cement to the exposed areas of the skull 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 to 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 using EMG electrodes 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 towards 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. (see **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 any other physiological signals of interest. In Figure 3, we monitor mystacial pad EMG and breathing (see **TROUBLESHOOTING**).

Optional Experimental Procedures

4) Perform any additional procedures required for the desired experiment at this point. In the example experiment shown in Fig. 5, we monitored neuronal activity in thalamus or cortex along with the vibrissa movement. In the example experiment shown in Fig. 6, we monitored facial motoneurons intracellularly, and in the example experiment shown in Fig. 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 hours are desired, cover the craniotomy with ACSF-soaked surgical foam. Suture the initial midline incision up to the dental acrylic which holds the head-restraint plate. Cover the skin around the plate with additional dental acrylic so that the surgical site is closed. Alternatively proceed direct to 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 hours post 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 2 times with PBS.

8) If you wish to viusalize BDA that was used as a tracer, add the solution containing streptavidin-Alexa-488 and incubate for 90 to 120 minutes. Rinse the sections 2-times 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.

TIMING

Equipment for holding head-restrained, lightly anesthetized rodents should be prepared at least one day prior to 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 making a craniotomy over the medulla, which takes approximately 40 minutes (Step 1, option A, steps i–v for rats; option B, steps i–vii for mice). The injection itself takes 30 to 40 minutes (option A steps vi–viii for rats; option B steps viii–x for mice. At this point, if the injection is successful, the animal can be prepared for head-restraint (Step 1, option A steps ix to xi for rats; option B steps xi to xii for mice) and attached to the head-restraining apparatus (Step 2), which takes another 40 minutes. Initializing the digital videography software (Step 3) takes 5–10 minutes. Preparation for additional measurements (Figs. 3, 5–7) can also be done at this time (Steps 2,4; time varies depending on 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 to 3 hours after the injection, and larger amplitude (10° to 30° peak to trough) movements after 2 to 4 hours. The movement continues for several hours even when the animal becomes alert after the anesthesia wears off. After the experiment the animal can be sacrificed, or the surgical site can be closed (Step 5, 15 minutes) and the animal can be placed in its home cage to recover. If the recording site is labeled (Figs. 3, 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 minutes) up to 72 hours 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.

TROUBLESHOOTING

See Table 1 for troubleshooting guidance.

ANTICIPATED RESULTS

Vibrissa movements are initially small, *i.e.*, < 2°, following the injection of kainic-acid and may only be observed on one or several vibrissae. In rats, continuous larger-amplitude rhythmic movement of 10° to 30° can be observed after 2 to 4 hours. In our hands, sustained vibrissa movements are obtained in approximately 75 % of injection attempts (see **TROUBLESHOOTING**). 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 while 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 to d is shown in Figure 3e,f. A three-dimensional reconstruction of the injection site in Figure 3e reveals that it is located in the intermediate reticular formation, dorsal to the pre-Bötzinger 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) three days 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 previously[13], 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 made a second craniotomy over VPM thalamus or vS1 cortex following the injection of kainic acid (**Protocol Step 1Aix for rat**). We then proceeded with **Protocol Steps 1Ax–xii, 2 and 3.** For **Protocol Step 4**, we lowered a pipette electrode into the thalamus or cortex using the micromanipulator. Figure 5a shows a juxtasomal 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 dye[52], and was located to 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

While it is known that whisking is controlled by motoneurons in the lateral division of the facial motor nucleus[53], the inputs to these motoneurons remain uncharacterized. The kainic-acid preparation allows us to stably record intracellularly from motoneurons using sharp pipette electrodes. 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 shows the membrane potential of a facial motoneuron along with kainic-acid induced vibrissa movements. Such recordings enable the temporal relationship between motoneuron post-synaptic 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 objects[54] or head turning[55]. 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 made in **Protocol Steps 1Av**. Immediately following the injection on one side (**Protocol Step 1Aviii**), a second injection was made on the contralateral side (**Protocol Steps 1Avi to viii**), and the motion of corresponding vibrissae on either side of the face 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.

CONCLUSIONS AND EXTENSIONS

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 predicable 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 to 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, since 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[60, 61].

Acknowledgments

This work was supported by grants from the National Institute of Neurological Disorders and Stroke (NS058668 and NS082097), the National Institute of Bomedical Imaging and Bioengineering (EB003832), the Canadian Institutes of Health Research (grant MT-5877), and the US-Israeli Binational Foundation (grant 2011432).

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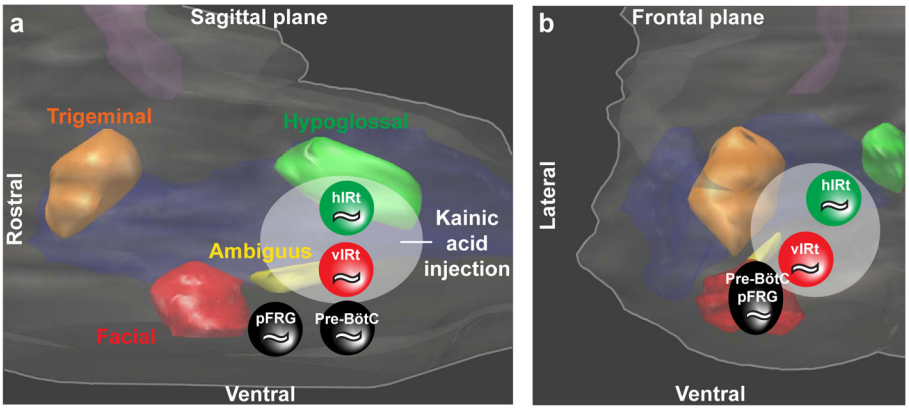

Figure 1. Target site for local injection of kainic acid to produce rhythmic vibrissa movements

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 (IRt) contains neuronal oscillators for licking (hIRt, green), whisking (vIRt, red), and breathing (black). The target injection site is shown in white in the sagittal **(a)** and **(b)** frontal planes.

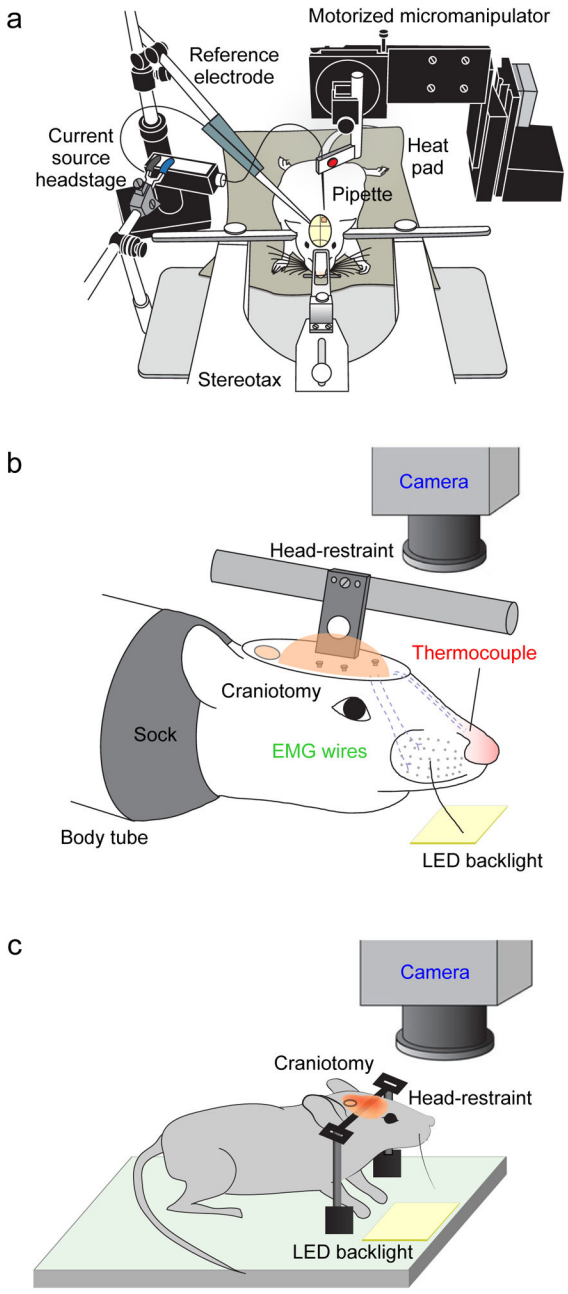

Figure 2. Diagram of experimental procedures to induce and measure kainic-acid induced vibrissa movements

(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 device 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 reference 13. **(c)** A

similar apparatus for mice. All animal procedures were approved by the IACUC at UC San Diego.

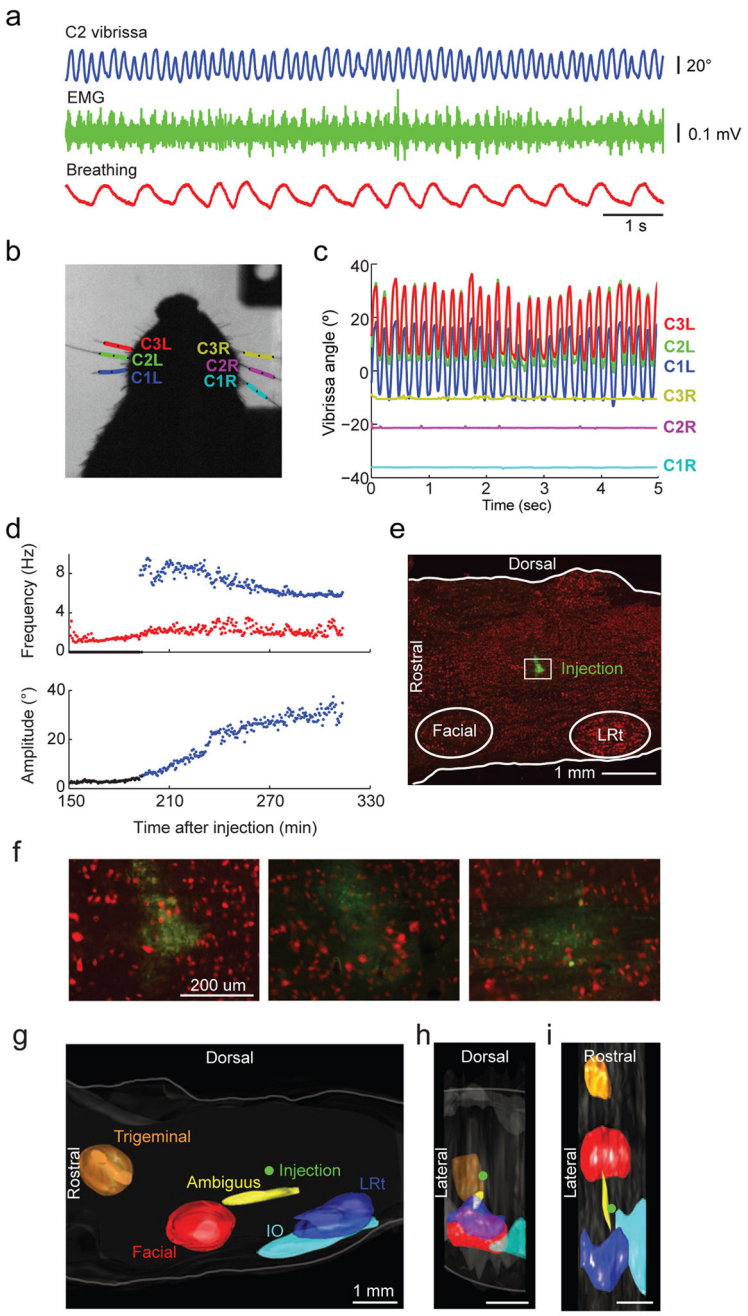

Figure 3. Kainic-acid injection produces rhythmic vibrissa movements in rat

(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 4 rats, all of which produced similar results. All rats represented in the present and subsequent figures were Long Evans females, 250 to 350 grams, 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 **panel b**, 5 hours post-

injection. Videography of multiple vibrissae was recorded in 8 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 $(1/2\pi)\cdot d\Phi(t)/dt$ averaged over 30 s intervals, where $\Phi(t)$ represents the instantaneous phase from the Hilbert transform of the vibrissa angle in time 31. The amplitude is defined as $2\cdot A(t)$ averaged over the same interval, where A(t) represents the amplitude of the Hilbert transform. The breathing frequency (top, red) is similarly defined. Frequency was defined only for movements that had an amplitude of greater than 5°/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 **Protocol Steps 16 to 20**, and counterstained with anti-NeuN. **(f)** Magnified view of the injection site in **panel 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 Data [13]. NeuN histology was performed on a total of 5 rats after labeling the injection site with BDA. Labeling was successful in 4 of the 5 attempts, and all 4 cases showed similar NeuN staining around the labeled site. **(g)** Sagittal view of a three-dimensional reconstruction of the injection site **in panel e** relative to anatomical landmarks: trigeminal (orange), facial (red), and ambiguus (yellow) motor nuclei, the inferior olive (IO, light blue), and the lateral reticular nucleus (LRt, dark blue). **(h)** Frontal and **(i)** horizontal views of the reconstruction in **panel g**. Reconstructions were made 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.

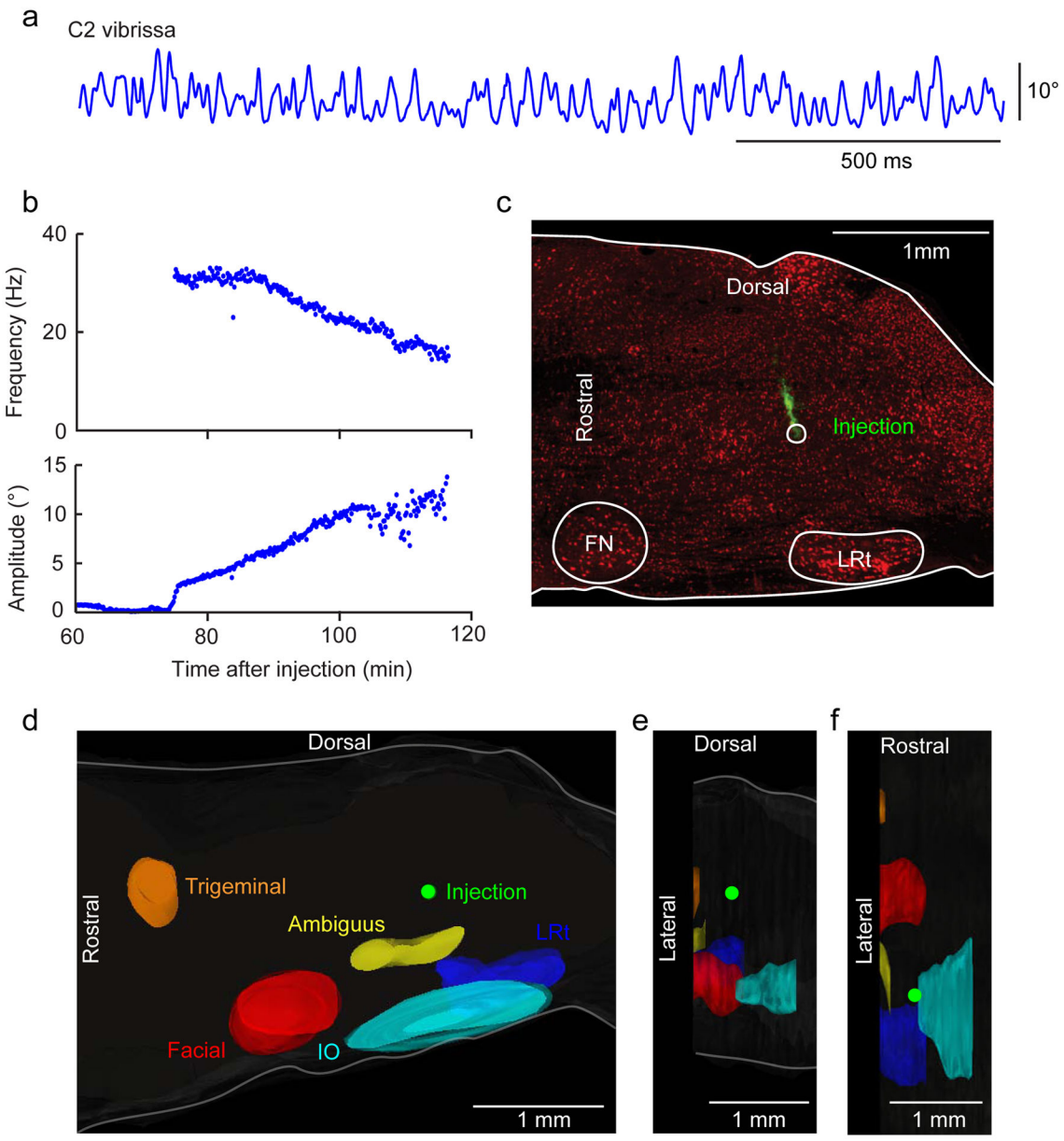

Figure 4. Kainic-acid injection produces rhythmic vibrissa movements in mouse

(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 C57Bl6 females, 20 to 30 grams, 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 5 mice after labeling the injection site with BDA. Labeling was successful in 5 of the 5 attempts, and all 5 cases showed similar NeuN staining around the labeled site. **(d–f)** Three-dimensional reconstruction of the injection site in **panel 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.

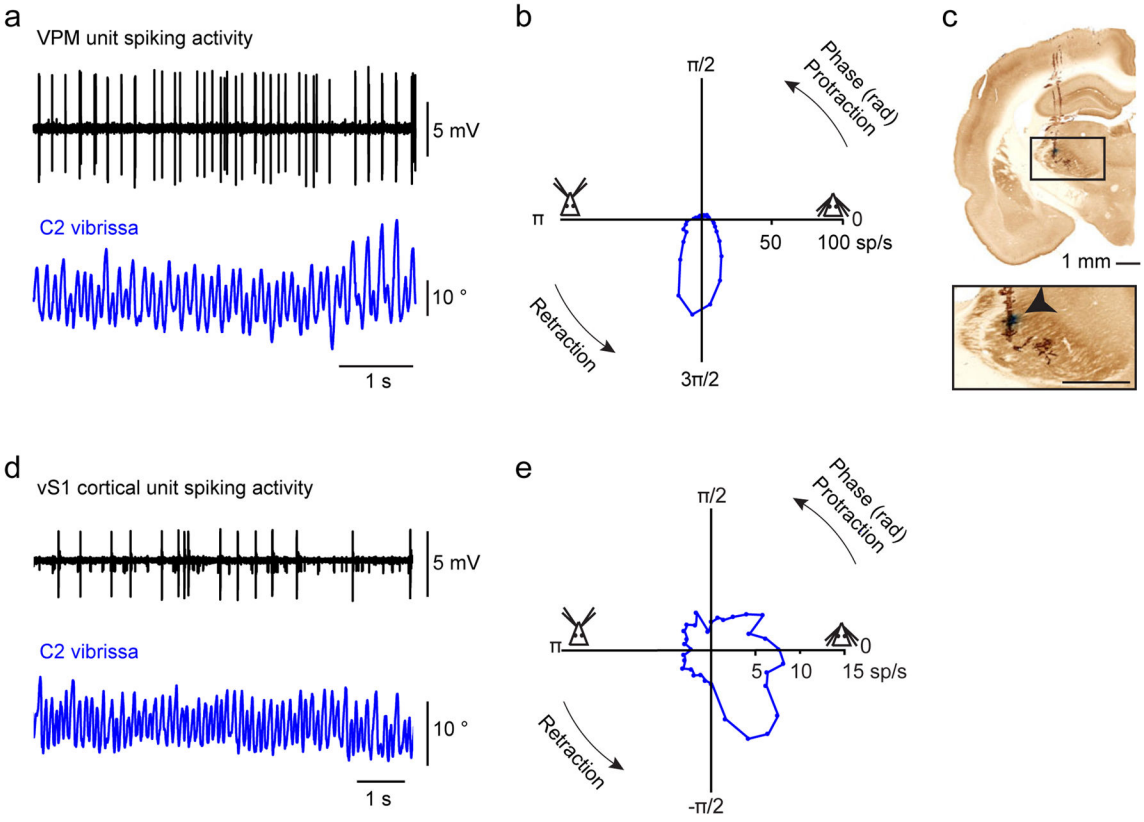

Figure 5. Juxtacellular recordings in somatosensory brain regions during kainic-acid induced vibrissa movements

(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 **panel a**. Instantaneous phase is defined using the Hilbert transform, as in Figure 3d. A total of 15 single units in or near VPM were recorded in 4 rats, 10 of which were significantly modulated by phase in the whisk cycle (Kuiper test[62, 63], $p<0.01$) **(c)** Anatomical location of the recording in **panels a,b.** The location was marked with Chicago sky blue dye[52], and the section was counterstained for cytochrome oxidase activity[64]. 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 **panels a,b,** respectively. A total of 10 single units were recorded in vS1 in 2 rats, 6 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.

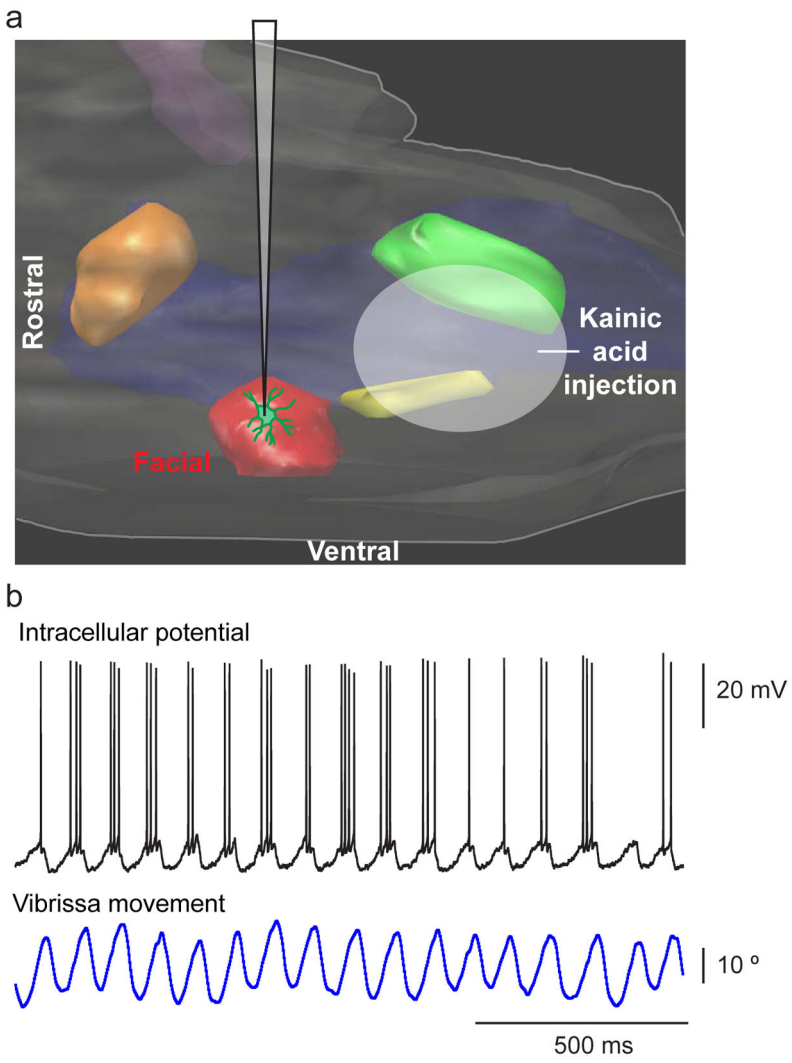

Figure 6. Intracellular recording in a facial motoneuron during kainic-acid induced vibrissa movements

(a) Schematic of the recording set-up. Conventions are as in Figure 1a. (b) Example record with membrane potential shown in black and vibrissa motion shown in blue. Similar recordings were obtained in a total of 9 cells in 4 rats. All animal procedures were approved by the IACUC at Laval University.

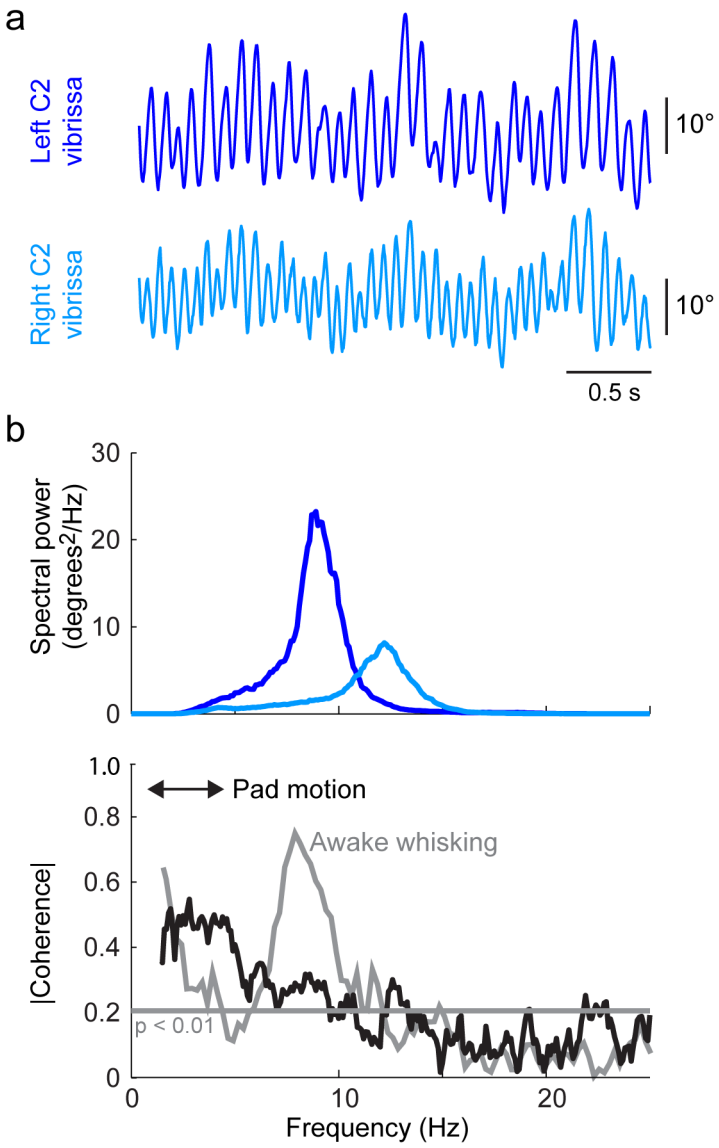

Figure 7. Bilateral kainic-acid injection produces independent vibrissa movements on the left and right sides of the face

(a) Movement of the left (dark blue) and right (light blue) C2 vibrissae following kainic acid injections. Similar results were obtained in a total of 2 rats. **(b)** Power spectra (top; dark and light blue) and spectral coherence (bottom; black) between the movements of each of the vibrissae in **panel a.** The two signals are 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.

Table 1

Troubleshooting

Step number	Problem	Possible reason	Solution
Equipment setup	Air-bubbles in pipette tip.	Solution does not wick properly.	Hold the pipette between thumb and forefinger and gently tap or flick it with the other forefinger. Alternatively, strike hand on a hard surface (table or lab bench), ensuring that the pipette does not directly contact with the surface.
1Avii or 1Bix	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 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.
1Avii or 1Bix	Breathing becomes irregular, or animal dies.	Too much kainic- acid is being injected, or the site is too close to critical respiratory centers.	There are several possible solutions. First, stop injecting current and wait 5 minutes while monitoring breathing. If breathing recovers to its regular rhythm, try moving the pipette dorsally 200 to 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 not inserted into muscle.	Re-fold the wires approximately 0.5 to 1 mm from the tips. Insert the needle as described in **Protocol 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 the forefinger while retracting the needle. This should hold the wires in place as the needle is being removed. CAUTION: Be sure 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.
3	Animal begins moving	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 (1/3 of initial dose); however, this may cause the amplitude of vibrissa movement to decrease.