Correlating membrane potential with behaviour using whole-cell recordings from barrel cortex of awake mice” by Sylvain Crochet and Carl Petersen.

Supplementary Methods

SUPPLEMENTARY METHODS

Animals and surgery
46 C57BL6J mice (1.5 to 2 month old; 21.4 ± 3.29 g), were used in this study. All procedures were carried out in accordance with protocols approved by the Swiss Federal Veterinary Office. Mice were anesthetised with isoflurane (1.5%). Paw withdrawal, whisker movement and eye blink reflexes were largely suppressed. A heating blanket maintained the rectally measured body temperature at 37°C. The head of the mouse was fixed by a nose clamp. The skin overlying the somatosensory cortex was removed and the bone gently cleaned. A metal bar for head-fixation and a recording chamber were glued on the skull of the animal. The recording chamber was centred on the left primary somatosensory cortex. Two macro-electrodes were inserted under the dura at the surface of the contralateral (right) somatosensory cortex and in the cerebellum respectively, for electroencephalogram (EEG) recording.

Training procedures
After two days of recovery, the implanted animals were progressively habituated to remain quiet while head-restrained. The habituation procedure consisted of daily sessions, whose duration increased every day to reach 1.5 hours. 6 to 10 days were necessary for the habituation, depending on the animal. After each session the animal was rewarded with sweet water. During the last 3 sessions, sham experiments were conducted to habituate the animal to experimental conditions and noise. The weight of the animal was monitored every day during the habituation procedure.
Targeting the C2 barrel with the intrinsic optical imaging

Once the animal had been habituated to the head-fixation procedure, a small craniotomy (< 1 mm in diameter) was made under isoflurane anesthesia to allow the insertion of the patch-pipette. The recording chamber was filled with silicone to protect the exposed brain from dust. The craniotomy was positioned just above the C2 barrel, whose location had been determined by intrinsic optical imaging.

Intrinsic signals were visualised through the intact bone covered with Ringer's solution sealed with a glass cover slip. The surface blood vessels were photographed using light of 530 nm to enhance contrast. The illumination was switched to 630 nm for functional imaging. The reflected light was imaged using a Qicam CCD camera (QImaging) coupled to a MZ9.5 stereo microscope (Leica). Image acquisition via a Firewire and stimulus control via an ITC18 (Instrutech) was governed by custom routines running in IgorPro (Wavemetrics). Alternating sweeps were imaged with or without stimuli delivered to the C2 whisker. Stimuli were applied at 10 Hz for 4 s using a piezoelectric bimorph attached to the C2 whisker and the intrinsic signal was quantified as the difference in the reflected light evoked by the stimulus. The intrinsic imaging resulted in localised signals centered on the C2 barrel column. This functionally identified location of the C2 barrel column was mapped onto the blood vessel pattern to guide surgery for the craniotomy.

Electrophysiology

After the craniotomy the animal was left in its cage to recover from the anesthesia. The mouse woke up in 5-10 min and started active behaviours (exploration, grooming). We waited an additional 2-3 hours before beginning the recording session to ensure a complete recovery. The animal was then positioned in the setup for patch-clamp recording and the craniotomy cleaned with Ringer’s solution. Pipettes were advanced into the cortex with a positive pressure until the pipette resistance increased and then suction was applied to establish a gigaseal followed by the whole-cell configuration. A stable
whole-cell recording was included in the data set only if the neuron had a membrane potential more hyperpolarised than -45 mV with overshooting APs.

Whole-cell pipettes had resistances of 5-7 MΩ and were filled with a solution containing (in mM): 135 potassium gluconate, 4 KCl, 10 HEPES, 10 phosphocreatine, 4 MgATP, 0.3 Na₃GTP (adjusted to pH 7.3 with KOH), and 2 mg/ml biocytin. Whole-cell electrophysiological measurements were made with a Multiclamp 700 amplifier (Axon Instruments, Foster City, California, USA). The Vm was filtered at 10 kHz and digitised at 20 kHz by ITC-18 (Instrutech Corporation, Long Island, New York, USA) under the control of IgorPro (Wavemetrics). Long sweeps of 300 s were recorded for the experiments using whisker magnetic stimulation combined with optic sensor whisker tracking. For the experiments using the high speed camera for whisker position tracking, only shorter sweeps could be recorded due to limitations in camera buffer memory.

**Filming and quantifying whisker behaviour**

Two techniques were used to monitor whisker movements. For experiments involving magnetic whisker stimulation a position sensing photodiode was used (Position Sensing Module and Versatile Position Sensing Amplifier, Laser Components). The photodiode can track a bright dot and return its XY coordinates as two voltage outputs. A small piece (1-2 mm in diameter) of reflective film (Diamond Grade, 3M) was glued to the C2 whisker of the mouse together with the metal particle for magnetic stimulation. A half-silvered mirror and lens was used to illuminate the reflector from the top and send the light from the reflector to the optic sensor. This technique allowed a rapid and simple online indication of whether the mouse was moving its whisker or not. However, because of difficulties with the calibration, it could not be used to measure whisker position. For quantitative measurement of the whisker position, we used a high speed camera to film the whisker of the mouse simultaneously with whole-cell recording. The behavioural area was illuminated from below with infrared light and filmed through a 50 mm video lens (Navitar) with a high speed MotionPro camera (Redlake). This
transillumination gave a high contrast silhouette of the mouse body and sufficient contrast to visualise the mouse whiskers. The field of view was 25x25 mm, with a single pixel resolution of 0.1 mm. The behavioural images were obtained at 2ms intervals between frames synchronised to the electrophysiological recording through a TTL pulse. Custom written routines running within ImageJ were used to automatically determine the whisker position (Loïc Segapelli, Sylvain Crochet, Isabelle Ferezou, Carl Petersen, Daniel Sage and Michael Unser, manuscript in preparation). The whisker position was measured frame by frame as the angle between the nose of the animal, the attachment of the whisker to the whisker pad and a point on the whisker. Because this technique requires the mouse to be illuminated from below, it is not compatible with the magnetic whisker stimulation for which an aluminium shield is needed between the coil and the animal to avoid electric noise. For these reasons, the optic sensor tracking was used in combination with magnetic whisker stimulation whereas the high speed camera was used to film the whisker for active touch experiments.

**Magnetic single whisker stimulation**

Because of spontaneous whisker movements in awake mice, it was not possible to use piezo-electric stimulation of the whisker. Therefore, we used a brief magnetic pulse to elicit a small and rapid single deflection of the C2 whisker transmitted by a small metal particle glued on the whisker. The animal was placed over an electromagnetic coil (10 cm inside diameter) and the right C2 whisker was positioned at the centre of the coil. During whisking, the whisker was moving perpendicularly to the magnetic field within an area of 2x2 cm. In this area the magnetic field was homogeneous. A short voltage pulse (1 ms, 100 V) was delivered to the coil and induced a brief magnetic pulse of 50 mT. The whisker deflection elicited by the magnetic pulse was in the range of few micrometers in the vertical axis (perpendicular to whisking movements). To avoid electric noise in the patch-clamp recording, it was necessary to place an aluminium shield between the electromagnetic coil and the animal.
Histology
After completion of the physiological measurements, the mice were deeply anesthetised by i.p. injection of a solution of 10% urethane (1.9 g/kg) and transcardially perfused with PBS followed by 4% paraformaldehyde. After overnight post-fixation, 100 µm thick tangential brain slices were cut using a vibratome. Slices containing barrel field in layer 4 were stained for cytochrome oxidase to reveal the barrel map. Then the slices were processed for biocytin staining using standard ABC kit (ABC-Elite; Vector Laboratories).
Neuronal reconstruction was performed using Neurolucida software (MicroBrightField). Soma, dendrites and axon were traced in three dimensions and merged from serially aligned sections. The barrel field in layer 4 was also traced with Neurolucida together the neuronal reconstruction.

Data analysis
Change in spontaneous activity
3s epochs during quiet periods and whisking activity were selected to compute the parameters of spontaneous activity for each behaviour. The mean Vm and its variance were computed after removing action potentials using a median filter. The Vm was not corrected for junction potential. The spectral analysis was done using a Fast Fourier Transform procedure under IgorPro. The FFT was computed after correction for the DC shift. The mean firing rate was computed for periods of spontaneous activity by counting the number of APs occurring for each behaviour (quiet and whisking) and dividing by the time spent in each behaviour.

Correlation between Vm and whisker position
Two methods were used to assess the correlation between Vm of cortical neurons and whisker movements. First we computed the cross-correlogram of the whisker position versus the Vm. The signals were band-pass filtered offline (1-1000 Hz). The cross-correlogram between the signals was computed using the equation from Lampl et al., 1999. This equation includes a correction for DC shifts and a normalisation of the signal amplitude using the standard deviation. The cross-correlograms were computed for each neuron for 3 s periods of whisking behaviour. The maximum absolute value of the
correlogram gave the time shift. The relative phase was calculated for each neuron as the ratio between the time shift and the mean length of the whisking cycle multiplied by $2\pi$ (polar plot in Fig. 1h). The sign of the peak gave the sign of the correlation (positive: open circles; negative: closed circles). The other way we assessed the correlation between Vm and whisker position was to compute the averaged Vm triggered from the peak of the whisker position (maximum protraction). The averaged trace revealed a peak that followed the maximum protraction. The modulation depth was calculated as the amplitude of the peak measured between the minimum (maximum for negative correlation) preceding the peak and the peak of the Vm average.

**Sensory evoked responses**

Sensory responses elicited by magnetic whisker stimulation were first sorted according to the mouse behaviour and then averaged to calculate the parameters of the response for each behaviour. The amplitude was measured as the difference between the mean Vm 2 ms before the stimulation and 2 ms at a fixed time point during the response (centered on the peak of the average response). The efficacy of sensory stimuli to evoke APs was evaluated as the difference in the number of APs 100 ms after and 100 ms before the stimulation, divided by the number of trials. For sensory responses evoked by active contact with an object the amplitude and efficacy to evoke an AP were calculated as for sensory responses elicited by magnetic whisker stimulation; except, the time window used to count APs after and before contact was only 50 ms to avoid contamination by successive contacts. The amplitude of individual responses was measured using the same reference times and this allowed the computation of the coefficient of variation (SD / mean). The latency was estimated by fitting the rising phase of the response with a sigmoid function (see Supplementary Methods Figure 1, below). The latency was taken as difference between the beginning of the contact and the time by which the fitted sigmoid curve reached 3% of its maximal amplitude. The time to peak was measured as the difference in time between the maximum of the evoked postsynaptic potential and the beginning of the contact. For each neuron we calculated the mean amplitude of the responses to 5 consecutive contacts from 3-7 whisking sequences (intervals between
consecutive contact onsets > 50 ms). The accommodation factor was computed as the amplitude of the response to the fifth contact expressed as a percentage of the amplitude of the response to the first contact.

Supplementary Methods Figure 1. Measurement of response latency.
(a) Responses to active touch in two different neurons. Individual sweeps were aligned to the onset of whisker-object contact, indicated by the red line. (b) The sweeps were median filtered to remove action potential waveforms and averaged (black trace). The period from whisker deflection to peak depolarisation was fitted by a sigmoidal curve (cyan curve). (c) The latency indicated by the blue dotted line was taken as the time where the sigmoid reached 3% of its maximal value. (d) The latency determined by deviation from linear fit (green dotted line) to the prestimulus
membrane potential trajectory is in close agreement with that obtained from the sigmoidal fitting procedure.

**Input resistance**

The change in input resistance ($R_{in}$) of cortical neurons was assessed by injecting square hyperpolarising current pulses of -100 pA through the recording pipette. Averaged responses to current pulses during periods of quiet behaviour and whisking behaviour were used to measure the change in $R_{in}$. Measurement of $R_{in}$ is complicated because of the difficulty in accurately correcting the access resistance. The difficulty results from the lack of distinct time-courses attributed to pipette access resistance and cell input resistance. Typically this correction is done during the experiment using the bridge balance. However, if access resistance is compensated during recording it must be continually reassessed and the precise value of the corrections continually noted. Alternatively the uncompensated voltage recording can be recorded and compensated off-line. This has the advantage that one can study the quantitative detail of the voltage trace kinetics, which might allow more accurate compensation to be performed. We chose to correct the access resistance offline (Supplementary Fig. 3). We first averaged all the current injections in the experiment. A single exponential was fitted to the voltage trace avoiding the first 10ms (during which the time-constants resulting from pipette access resistance will dominate). The exponential curve was back extrapolated to the onset time of the current injection and the value at this time provided an estimate of the access resistance. This voltage difference attributed to access resistance was subtracted to obtain the corrected Vm trace. The $R_{in}$ was then calculated using the difference in corrected Vm between the baseline (100 ms before the current pulse) and the steady state (100 ms at the end of the current pulse). This value is an underestimate of the absolute value of the $R_{in}$ since the cellular membrane time-constants will also contribute to the first 10ms of the current injection, which in this analysis we attribute entirely to the pipette access resistance. However, the reported decrease in $R_{in}$ during whisking behaviour is a robust observation and the difference is significant irrespective of whether access
resistance is compensated or not. The relative importance of the $R_{in}$ reduction during whisking does depend on the access resistance correction.

Reference