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Sparse, reliable, and long-term stable representation of periodic whisker deflections in the mouse barrel cortex

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ABSTRACT

The rodent whisker system is a preferred model for studying plasticity in the somatosensory cortex (barrel cortex). Contrarily, only a small amount of research has been conducted to characterize the stability of neuronal population activity in the barrel cortex. We used the mouse whisker system to address the neuronal basis of stable perception in the somatosensory cortex. Cortical representation of periodic whisker deflections was studied in populations of neurons in supragranular layers over extended time periods (up to 3 months) with long-term two-photon Ca^{2+} imaging in anesthetized mice. We found that in most of the neurons (87%), Ca^{2+} responses increased sublinearly with increasing number of contralateral whisker deflections. The imaged population of neurons was activated in a stereotypic way over days and for different deflection rates (pulse frequencies). Thus, pulse frequencies are coded by response strength rather than by distinct neuronal sub-populations. A small population of highly responsive neurons (~3%) was sufficient to decode the whisker stimulus. This conserved functional map, led by a small set of highly responsive neurons, might form the foundation of stable sensory percepts.

Introduction

The somatotopic mapping of individual whiskers to specific areas of the somatosensory cortex is one of the reasons why the whisker system is widely used to study cortical plasticity. For example, whiskers have been cut or removed to study map reorganization (Diamond et al., 1994; Feldman, 2005; Margolis et al., 2012). On the other hand, to date, only a small amount of research has been conducted to characterize the persistency of cortical stimulus representations at the single-cell level (Lütcke et al., 2013) (however, see Masino and Frostig, 1996; Minderer et al., 2011) for studies measuring wide-field signals and (McMahon et al., 2014) for extracellular recordings from individual neurons over months in non-human primates). Plasticity enables us to learn new experiences and adapt to new stimulus conditions, whereas stability of stimulus representation enables us to recognize familiar stimuli even under changing conditions.

Rodents are nocturnal animals and rely on a highly sensitive whisker system. This tactile sense is essential for navigation and object recognition. Rodents are able to actively (whisking) and passively use their whiskers to solve different kinds of tasks, such as roughness discrimination, gap crossing, pole detection, and frequency discrimination tasks (Adibi et al., 2012; Carvell and Simons, 1990; Gerdjikov et al., 2010; Hutson and Masterton, 1986; Jenkinson and Glickstein, 2000; Knutsen, 2006; Mayrhofer et al., 2013; Mehta et al., 2007; Morita et al., 2011). In the somatosensory cortex (S1), inputs from the contra- and ipsilateral whisker pads converge for the first time in the ascending somatosensory pathway (Shuler et al., 2001; Wiest, 2005).

One way to reliably activate the whisker pathway is by rapid changes of the whisker's position (stick-slip events) which correspond to high velocity deflections (Jadhav et al., 2009; Lottem and Azouz, 2009; Wolfe et al., 2008). The frequency of successive stick-slip events – created either by active whisking against a rough surface or by passive deflection when the animal runs along a wall – characterizes the texture of a surface (Arabzadeh et al., 2005). Key questions are how different event frequencies are processed in the barrel cortex and whether simultaneous bilateral activation alters these processes. Whiskers could act as resonators, with each whisker having a distinct characteristic frequency (100–700 Hz). The spatial organization of the whiskers on the rodent's snout would directly translate to frequency representation (Andermann et al., 2004; Moore, 2004). On the other hand, barrel cortex neurons show stimulus locking up to frequencies as high as 1000 Hz (Ewert et al., 2008). This means that a temporal code could be used for differentiating different

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frequencies. Alternatively, integrated spike counts have been proposed as a way for the animal to discriminate frequencies (Musall et al., 2014; Stüttgen and Schwarz, 2010).

The present study investigates three main questions: 1) how are different whisker deflection frequencies (pulse frequencies) represented by populations of neurons in the barrel cortex (single barrel); 2) how stable is whisker stimulus representation over time in terms of neuronal population responses; and 3) how stable is the classification accuracy of these stimuli over extended time periods?

Materials and methods

Animals

In total, seven female adult mice (strain: C57BL6J; weight: 20–26 g; Harlan Laboratories, Netherlands) were imaged. The age of the animals on the day of head post implantation was >14 weeks. Mice were housed individually, with food ad libitum and under an inverted 12-hour light/dark regime. All surgical and experimental procedures were approved by the local veterinary authorities, conforming to the guidelines of the Swiss Animal Protection Law, Veterinary Office, Canton Zurich (Act of Animal Protection 16 December 2005 and Animal Protection Ordinance 23 April 2008).

Head post implantation, virus injection, and cranial window implantation

The animals were anesthetized with isoflurane (1–3%; Abbott, USA). The depth of anesthesia was checked on the basis of hindpaw withdrawal and corneal reflexes. The animal's temperature was maintained at 37 °C by a feedback-controlled heating pad in combination with a rectal temperature probe (Harvard Apparatus, USA). To protect the eyes from desiccation, an ointment was applied (vitamin A eye cream; Bausch & Lomb, Switzerland). Preparation of the animals was divided into two separate surgeries (1–3 weeks apart). In the first surgery, a stereotaxic apparatus was used to fix the animal. Once the skull was cleaned, it was moistened with a water-based gel (Skintact Ultrasonic Gel; Leonhard Lang GmbH, Austria) and a window was positioned at the estimated region of the barrel cortex (1 mm caudal and 3 mm lateral from Bregma) to perform intrinsic optical imaging (IOI; Fig. 1B) with a 630-nm illumination (Margolis et al., 2012). After functionally identifying the barrel cortex, a bonding agent (Gluma Comfort Bond; Heraeus Kulzer, Germany) was applied to the cleaned skull and polymerized (blue light, 600 mW/cm^2; Demetron LC, Switzerland). In order to sculpt the headcap, several layers of transparent light-curing dental cement (Tetric EvoFlow; Ivoclar Vivadent AG, Liechtenstein) were placed on top of the bonding layer. The area of the left barrel cortex was spared for the ensuing virus injection and window implantation. A custom-made aluminum head post was attached (2 mm caudal to Lambda) with additional dental cement. After washing the wound with saline, an antibiotic ointment was applied (Cicatrex; Janssen-Cilag AG, Switzerland). The open skin was sutured and attached to the implant with acrylic glue (Histoacryl; B. Braun, Germany). In the second surgery, a craniotomy above the left barrel cortex was performed (approx. 4 × 4 mm). Virus was injected through thin glass pipettes (Cetin et al., 2007) to the principal whisker area identified by IOI. 150 nl of rAAV hybrid serotype 2/1 (40 nl/min) carrying the Yellow Cameleon 3.60 (YC3.60, under human synapsin promoter) construct was injected at 300-μm depth below the dura mater. To facilitate infection efficiency and spread (Mastakov, 2001) we coinjected a hypertonic D-mannitol solution (1:2, Mannitol 20%; B. Braun, Switzerland). The human synapsin promoter was used to selectively express YC3.60 in neurons (Lütcke, 2010). A square cover slip (3 × 3 mm; UQG Optics Ltd, UK) was lightly pressed on the exposed brain and fixed with dental cement to the headcap. Following the surgeries, the animals were kept warm and provided with analgesics (Novaminsulfon, 50%; Sintetica, Switzerland). During the first week of recovery, an antibiotic was added to the drinking water (Baytril (enrofloxacin), 200 mg/l drinking water; Bayer, Germany).

Whisker stimulation

A single whisker was plugged into a glass capillary (GB 120-8P; Science Products GmbH, Germany; tip was melted to reduce dead space), which was mounted on a piezo actuator (T223-H4CL-303X; Piezo Systems, USA). The control voltage driving the piezo actuator was generated by a custom-written LabVIEW program (National Instruments, USA) using a multifunctional data acquisition card (PCI-6229; National Instruments) and was amplified by a piezo controller (MDT693A; Thorlabs, USA). The amplitude of the stimulator was calibrated using a laser displacement sensor (ILD1700-2; Micro-Epsilon, Germany) (see also Mayrhofer et al., 2013, for an example of recording of stimulus).

The stimulus consisted of a series of deflections to a single whisker (identical whisker for the entire recording period) on the animal's right whisker pads. The deflection was in the anterior–posterior (rostral–caudal) direction. Whiskers were slightly cut to ensure reliable and reproducible single whisker stimulation over days. Depending on the IOI map and the virus expression area, different principal whiskers were used in different animals. The peak velocity of a prototype pulse (taken from a single period 120-Hz cosine wave; peak-to-peak amplitude was 120 μm) was kept constant and the repetition rate of pulses was varied (10, 40, 90, and 110 Hz) on the contralateral side with respect to the imaging side. The stimulus duration was 1 s. The stimulator was placed 4 mm away from the whisker pad and led to a peak-to-peak amplitude of 1.72° and maximum velocity of 648°/s. The stimulus sets were presented in a randomized order within an imaging day to minimize the influence of changes in anesthesia (inter-stimulus intervals: 10–14 s). Each stimulus was presented at least 30 times per imaging day.

Two-photon imaging settings

A 20× water immersion microscope objective was used (W Plan-Apochromat 20×/1.0 DIC VIS-IR; Zeiss, Germany). The genetically encoded Ca^{2+} indicator YC3.60 was excited with a Ti:sapphire laser (870 nm, 140-fs pulses at 80 MHz, Chameleon Ultra II; Coherent, USA). Emission of YC3.60 was detected with two GaAsP photomultiplier modules (H10770PA-40; Hamamatsu Photonics, Japan) with the following filter settings: dichroic mirror 515 DCXR, band pass filter BrightLine HC 542/50 (yellow channel), band pass filter BrightLine HC 475/64 (cyan channel) (Semrock, USA), and short pass filters BrightLine 750/sp for each channel. The two-photon laser-scanning microscope was controlled by custom-written LabVIEW software (National Instruments) (Langer et al., 2013) or Scanimage (Pologruto et al., 2003) running on personal computers using multifunctional data acquisition cards (National Instruments).

Chronic two-photon imaging

Chronic imaging started at the earliest at three weeks after virus injection using a custom-built two-photon laser-scanning microscope (see two-photon imaging settings). Functional signals (128 × 128 and 256 × 128 pixels) were acquired at a frequency of 8-Hz and 11.84-Hz bidirectional scan, respectively. Anatomical imaging was performed with a resolution of 256 × 256 or 512 × 512 pixels. Recordings were performed under isoflurane anesthesia (0.7–1.1%; Abbott). The respiratory rate was monitored with a piezo element (7BB-35-3; Murata Manufacturing Co., Ltd., Japan) attached to an oscilloscope and was maintained at around 150 bpm by adjusting the anesthesia level. A correlation analysis of spontaneous activity (0 Hz, Ca^{2+} response probability) of one session with the previous session (Margolis et al., 2012) showed that Ca^{2+} response probability ($\rho = 0.345$ $p < 0.001$) was significantly correlated. Hence, spontaneous activity was similar over

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