NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

S. I. Shumikhina et al. / Neuroscience xxx (2018) xxx–xxx

# Dynamics of Stability of Orientation Maps Recorded with Optical Imaging

S. I. Shumikhina,[a]* I. V. Bondar[b] and M. M. Svinov[a]

[a] Functional Neurocytology, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, 5a Butlerova Street, 117485, Russia

[b] Sensory Physiology, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, 5a Butlerova Street, 117485, Russia

**Abstract—Orientation selectivity is an important feature of visual cortical neurons. Optical imaging of the visual cortex allows for the generation of maps of orientation selectivity that reflect the activity of large populations of neurons. To estimate the statistical significance of effects of experimental manipulations, evaluation of the stability of cortical maps over time is required. Here, we performed optical imaging recordings of the visual cortex of anesthetized adult cats. Monocular stimulation with moving clockwise square-wave gratings that continuously changed orientation and direction was used as the mapping stimulus. Recordings were repeated at various time intervals, from 15 min to 16 h. Quantification of map stability was performed on a pixel-by-pixel basis using several techniques. Map reproducibility showed clear dynamics over time. The highest degree of stability was seen in maps recorded 15–45 min apart. Averaging across all time intervals and all stimulus orientations revealed a mean shift of 2.2 ± 0.1°. There was a significant tendency for larger shifts to occur at longer time intervals. Shifts between 2.8° (mean ± 2SD) and 5° were observed more frequently at oblique orientations, while shifts greater than 5° appeared more frequently at cardinal orientations. Shifts greater than 5° occurred rarely overall (5.4% of cases) and never exceeded 11°. Shifts of 10–10.6° (0.7%) were seen occasionally at time intervals of more than 4 h. Our findings should be considered when evaluating the potential effect of experimental manipulations on orientation selectivity mapping studies.** 

**Key words: visual cortex, intrinsic optical imaging, orientation selectivity, variability.**

## INTRODUCTION

Orientation selectivity is a characteristic feature of visual cortical neurons. Neurons tuned to similar orientations are grouped together into columns perpendicular to the cortical surface and extending to the white matter, with cross-sectional diameters at the surface on the order of 0.5 mm (Hubel and Wiesel, 1962). The preferred orientation varies continuously, parallel to the cortex, forming orientation maps. Pinwheel-like patterns of orientation preference in cat visual cortex form an irregular mosaic of modular units with an average density of 1.2 pinwheels per square millimeter (Bonhoeffer and Grinvald, 1993). Optical imaging recording is a technique that is widely used to study orientation selectivity of visual cortical neurons. It is based on metabolically induced changes that occur in microcirculation (Frostig et al., 1990). Optical imaging of the visual cortex allows for the visualization of the activity of large populations of neurons and the construction of spatial maps of orientation selectivity (Frostig et al., 1990; Ts'o et al., 1990; Shmuel and Grinvald, 2000).

Determining the stability of orientation maps is very important, but has not yet been systematically investigated. Typically, the effect of an experimental condition on the orientation map is estimated immediately after being introduced, and then again after some time has transpired. The time interval between orientation maps is often an hour or more, in order to confirm the significance of the effect by comparison between control and recovery conditions, as in the case of pharmacological, electrical stimulation, or other interventions. Although the reproducibility of orientation preference maps has been described by several authors (Bonhoeffer and Grinvald, 1993; Shtoyerman et al., 2000; Godde et al., 2002; Sharon and Grinvald, 2002; Bachatene et al., 2015), no systematic investigation examining the stability of orientation preference maps over sufficiently long periods of time has been reported to date.

There are two principal techniques for recording intrinsic signal optical responses. One is based on interleaved presentations of visual stimuli, usually of 4–8 orientations presented several times in a random order.

*Corresponding author. Fax: +7-499-743-0056.
E-mail addresses: shumikhs3@yahoo.com (S. I. Shumikhina), bondar_iv@inbox.ru (I. V. Bondar), svinov@ihna.ru (M. M. Svinov).
*Abbreviations:* ROI, region of interest.

https://doi.org/10.1016/j.neuroscience.2018.01.030
0306-4522/

To produce the cortical map and to correct for noise from various sources, this method uses averaging of stimuli for each orientation, with normalization to a "blank" or "cocktail blank" stimulus (Bonhoeffer and Grinvald, 1993). Another method, employing a continuous-periodic stimulation combined with continuous data acquisition, was developed by Kalatsky and Stryker (2003). The latter technique uses Fourier analysis of the continuous data stream for effective separation of the stimulus-evoked responses from intrinsic noise. Artifacts such as heart beat (2–5 Hz), respiration (0.3–1 Hz), and vasomotor signal (0.05–0.1 Hz) (Mayhew et al., 1996; Kalatsky and Stryker, 2003) are removed if the frequency of stimulation is between 0.1 and 0.3 Hz, or below 0.05 Hz (Kalatsky and Stryker, 2003). This method also has the advantage of being much faster, resulting in a 30-fold or greater reduction in acquisition time (Kalatsky and Stryker, 2003).

In the present study, we were interested in assessing the stability of orientation maps recorded at various time intervals from 15 min to 16 h. Our study was aimed at the analysis of orientation maps recorded with continuous-periodic stimulation combined with continuous data acquisition. We are not aware of any previously published studies offering a quantitative description of the map's variability recorded by this method. However, it appears to be important to know the statistical limits of the orientation changes under normal conditions, especially given that subtle effects may be observed after experimental manipulations. As our study was aimed at the problem of reproducibility of orientation maps, eliminating noise in the recordings made our results more reliable. We observed that map reproducibility showed clear dynamics over time.

## EXPERIMENTAL PROCEDURES

### Animal preparation

Five adult cats were used in the experiments. Animal preparation and recording procedures followed Directives 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, and were also approved by the Animal Care and Use Committee of the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences.

Animals were initially anesthetized with ketamine hydrochloride (Calypsol, Gedeon Richter Plc., Budapest, Hungary; 10–15 mg/kg, i/m) in combination with vetranquil (Ceva Santé Animale, Libourne, France; 30–40 mg/kg). A tracheotomy was performed for artificial ventilation, and one forelimb vein was cannulated. For the remaining preparations and recording, paralysis was induced with arduan (Gedeon Richter Plc., Budapest, Hungary, 0.2–0.4 mg/kg, i/v) in 5% dextrose lactated Ringer's nutritive solution. Arduan (02–0.4 mg/kg) was also administered every 1.5–2 h for additional animal relaxation.

During the experiment, propofol (Diprivan, Fresenius Kabi, Bad Homburg, Germany; mg/kg, i/v) was used as a general anesthetic, delivered continuously at 2–4 mg/kg/h, in order to maintain a constant and controlled depth of anesthesia. In addition, a subcutaneous injection of butomidor (Richter Pharma AG, Wels, Germany; 2–4 mg/kg) was administered every 6 h to reduce pain. The anti-inflammatory agent dexamethasone (Shreya Life Sciences Pvt. Ltd, Mumbai, India; 2–4 mg/kg) was injected subcutaneously every 12 h. Functional condition of the animal was controlled continuously by monitoring $CO_2$ concentration in the expired air (3.8–4.0%), level of blood oxygenation (99.0%), heart rate (110–140 beats/min), body temperature (38.5 °C) and electroencephalogram. The head of each animal was set in the stereotaxic apparatus with ear, mouth, and orbital bars. The orbital bar pressed lightly on the eyeball, restricting its movement. Pupils were dilated with atropine (1%) and the nictitating membranes were contracted with phenylephrine hydrochloride ophthalmic solution (Iriphrine, Sentiss Pharma Private Limited, Haryana, India; 2.5%). Plano contact lenses were placed on the cat's eyes to protect the cornea from drying. One eye was closed with a non-transparent partition. Additionally, the eyelids were opened with a lid retractor, which also restricted eyeball movement.

The skull was opened with a trepan (16 mm diameter) over the visual cortex (areas 17 and 18) of both hemispheres. After durotomy, the opening was covered with agarose (Fluka Biochemicals, Sigma–Aldrich; 3%) with a low temperature of hardening. A cover glass was placed above the agarose.

### Visual stimulation

Monocular stimulation under scotopic adaptation with moving clockwise square-wave gratings that continuously changed orientation and direction was employed (method of Kalatsky and Stryker, 2003). Visual stimuli (100% contrast, 0.2 cyc/deg, 2 cyc/s, 1 min/cycle, 360° /min rotation) were generated by custom-made software (ContStim developed by Dr. V. Kalatsky) and displayed on a cathode ray screen (LG Flatron 795RT Plus, effective display area of 305 × 235 mm, mean luminance 40 cd/m$^2$, with a refresh rate of 70 Hz) placed 57 cm from the cat's eyes, centered on the representation of the area centralis and synchronized with the data acquisition processes.

### Optical imaging

Optical imaging of intrinsic signals was performed using the imaging system and custom-made data acquisition software developed by Dr. V. Kalatsky. The intrinsic optical signal was recorded using a CCD camera (Dalsa 1M60P, USA) with 1024 × 1024 pixels in a matrix of 12 × 12 mm. Frames were acquired at the rate of 30 frames per second. Binning by four frames temporally reduced the sampling rate for the stored images to 7.5 Hz. The frames were stored as 512 × 512 pixel images after binning by 2 × 2 pixels spatially. The pattern of surface blood vessels of the brain was first captured under green illumination (546 nm). The camera was then focused 600–700 μm below the cortical surface to