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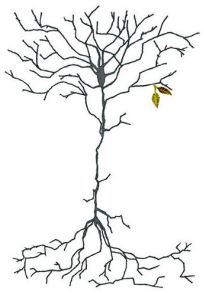

The spatial structure of surround modulation in mouse visual cortex

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Thesis to obtain the Master of Science Degree in

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Prof. Lorem Ipsum

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We are so familiar with seeing, that it takes a leap of imagination to realize that there are problems to be solved. But consider it. We are given tiny distorted upside-down images in the eyes, and we see separate solid objects in surrounding space. From the patterns of stimulation on the retina we perceive the world of objects and this is nothing short of a miracle.

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Abstract

The Objective of this Work ... (English)

Keywords

Keywords (English)

Resumo

O objectivo

Palavras Chave

Palavras-Chave

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Developing precise and reliable tools for the spatial and temporal mapping of neuronal activity is crucial to understand the functional architecture of the brain. Radiotracer, electrophisiological, magnetic resonance, anatomic and optical imaging techniques all offer advantages and disadvantages to this end [REFERENCES]. On its part, optical imaging of neuronal activity allows the mapping of large regions of the cortex, varying in time as responses to stimuli. This can be acomplished with voltage sensitive dyes [REFERENCES], changes in the optical properties of the tissue or with the aid of genetic tools. In here, we review the utilized techniques: Intrinsic optical signal imaging that draws on the changing reflectance in the hemodynamics of the cortex and two-photon laser microscopy that takes advantage of genetic manipulation tools.

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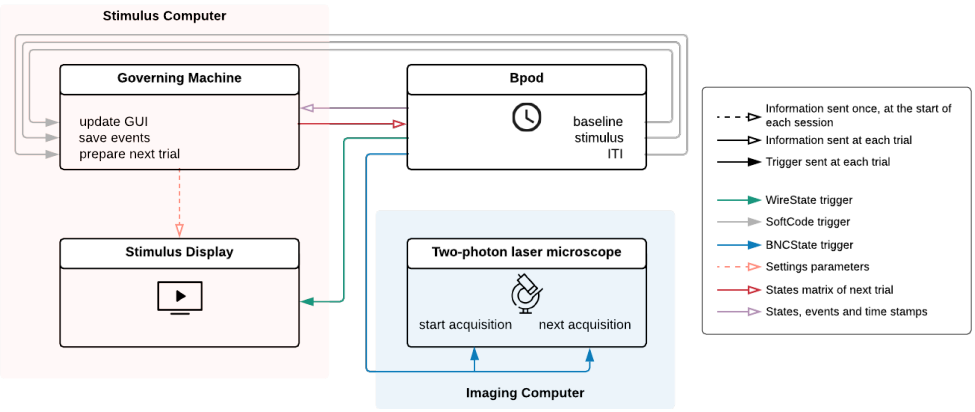

Figure 4.1: Setup and connections scheme of devices used for stimuli presentation and real-time imaging.

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Over the experimental process, the appropriate settings of both the animals' state of alertness and of the visual stimulation were chosen as an operative practical balance. In this chapter we describe the methods and specifications of the animals' care and craniotomy cirurgies, as well as the settings of the ISOI and visual stimulation sessions.

5.1 Animals

All procedures were approved by the Champalimaud Centre for the Unknown Ethics Committee and carried under the stipulations of the Portuguese Direção Geral de Veterinária. Mice were held in individual cages on a reversed light-dark cycle with access to food and water. Mice were exclusively used for the experiments regarding this thesis' work.

Cells' somata in V1 layer 2/3 of four Thy1-GCaMP6s ?? male?? mice (?? Laboratory stock no:??) were imaged.

Prior to the imaging experiments, once adults (?? to ?? weaks old), the mice underwent chronic window implantation surgeries. A circular craniotomy of diameter $4mm$ was performed over each mouse's left visual cortex, leaving the dura intact. The imaging windows were constructed using two layers of microscope cover glass (Fisher Scientific, no. 1 and no. 2) and UV-curable optical glue. A window was placed into the craniotomy using black dental cement and an iron headpost was attached to the skull with dental acrylic. The subjects were kept under isoflurane anesthesia, as well as Bupivacaine (0.05%; injected under the scalp) and Dolorex ($1mg/kg$; injected subcutaneously), serving respectively as local and general analgesia. Eye moisturing was insured with ophtalmic ointment (Clorocil, Laboratorio Edol).

For both the ISOI and visual stimuli protocols, the animals were lightly anesthetized with isoflurane (1%) and injected intramuscularly with chlorprothixene (1mg/kg), a muscular paralyzer to circumvert the need for higher anesthesia concentrations which could depress the recorded neuronal responses. Mice were headfixed by the headposts during all of the visual stimuli presentations and their eyes were protected and kept moist with silicone oil (Sigma-Aldrich) in thin, uniformly coated layers.

5.2 Visual stimulation

For both the ISOI and visual stimuli protocols, an LED display (BenQ XL2411Z, 144-Hz monitor, stimulus presented at $60Hz$) was used. The screen was placed at $15cm$ from the mouse's right eye and aligned at 30^o to the axis of its nose-line, insuring access to the visual space of 120^o in azimuth and 60^o in elevation???. The stimuli were produced and presented using Matlab and the Psychophysics Toolbox [REFERENCES] (chapter 3,??).

5.3 Intrinsic signal optical imaging settings

The course of action with each craniotomized mouse started with the performance of intrinsic signal optical imaging (ISOI) over the mice's primary visual cortex and surrounding visual areas [REFERENCES] to obtain a reasonable spatial resolution retinotopic mapping of the temporaly dependent

hemodynamics of the accessed brain while moving visual stimuli was presented to the animal on a monitor aligned to the center of the mouse's right eye and forming a 30^o angle with the animal's nose-line.

The stimuli consisted of a checkerboard of alternate flickering light/dark squares ($5Hz$) that was masked to continuously expose only a periodic drifting stripe of the grid in four consecutive cardinal directions ($12s$ period, 20^o width, 80 times for each direction) - an horizontal stripe going from the top to the bottom of the screen, vice-versa, a vertical stripe going from its left to its right or in the opposite direction.[GET IMAGE]

The cortical surface of an head-fixed mouse was illuminated with a $620nm$ red LED to allow the intrinsic hemodynamic signals to be recorded as optical images of reflectance change correspondent to cortical activity. This recording was held using a Retiga QIClick camera (QImaging) controlled with Ephus[REFERENCE] with a high magnification zoom lens (Thorlabs) at $5Hz$ focused under the cranial window, at the brain surface. A $535nm$ green LED was also used to obtain an image of the cortical vasculature.

For each animal, this resulted in a retinotopical map of 512×512 pixels, representing ??×?? of cortical area. This corresponded to the color-map of cortical encoding of both azimuth and elevation stimuli locations, superimposed on the image of the mouse's vasculature[GET IMAGE]. These correspondence figures were subsequently used as a first-approach guide to encountering the V1 positions aimed for imaging - those whose neurons responded to stimuli in the center of the mouse's visual field where the central stimuli were displayed during the following two-photon microscopy imaging sessions.

5.4 Session and trial structure

The experimental process of a session comprised three main protocols for each mouse and each of that animal's V1 imaged position: A protocol to establish the receptive fields of the imaged neurons (`StimPresProt_RF`), another to regard their tuning properties (`StimPresProt_tuning`) and a last protocol designed for the actual surround modulation examinations (`StimPresProt_RF`).

Each protocol involved a pseudorandomized sequence of trials - N repetitions of X trial types. Repetitions of each stimulus type are required in order to enhance the signal to noise ratio of the responses by trial averaging.

In general, each trial was formed by an initial baseline, a stimulus presentation, and an inter-trial interval (ITI). In both the baseline and the ITI the screen was left at background brightness and contrast level (grey) and its duration was used as buffer time for internal computations and to ensure sufficient Calcium decay from the previous stimulation (from the previous trial in the case of the baseline, and from the same trial in the case of the ITI). A session's total stimuli display duration should not be longer than two hours, as the anesthesia produces cumulative effects in the central nervous system and can start depressing the neuronal responses, impeaching the subsequent study of its relation with the visual stimulation [REFERENCES]. Thus, the durations of these intervals depended on the specific protocol (chapter 4, section d), as a balance between how important was the separation of responses in between trials - the more precise the intended separation, the larger should be the baseline and ITI

durations - and how many trial types and trial repetitions were intended - the more trials, the less duration the baseline and ITI should have.

	Number of trial types	Number of repetitions	baseline (ms)	stimulus (ms)	ITI (ms)
RF	80	14	0	880	120
tuning	32	25	5	900	95
SM	124	15-20	500	1000	500

Table 5.1: Protocol configurations regarding session extension and trial durations.

5.5 Specifics of the protocol settings and visual stimuli

In a session, the three stimuli presentation protocols were ordered as RF mapping, tuning mapping and then the actual SM examination. Each protocol is associated with different stimuli characteristics, specific to the controls or information that were to be required from the final extracted data. Furthermore, each kind of protocol had also distinct specifications in regards to the time durations in the trial structure of the session. The mice were always placed with their right eye parallel and at $15cm$ from the center of the monitor. All of the stimuli measurements will thus follow indicated in degrees at the mice's perspective to the screen, in azimuth (horizontal axis) and in elevation (vertical axis) coordinates. In addition, to compensate for the screen's flatness, spherical corrections were applied to the displayed stimuli, so that what the mice visualized corresponded to the same size of stimuli at each patch location, irrespective of its distance in the screen and that no distortions in the gratings were perceived by the animal. Every protocol underwent pseudorandomization of the trials: Each type of trial appeared the same number of N repetitions, but at shuffled order. The reason for this was to minimize the neurons adaptation [REFERENCES] to the specific trial types, as they had to be repeated a reasonable amount of times for significant analysis.

5.5.1 Receptive Field mapping stimuli

This protocol consisted on the presentation of a 10^o squared cell (in the mouse's referential) with a small moving bar inside. At each trial, this bar moved in four directions, in sequence but at random order - bottom to top (labelled 0^o), left to right (90^o) and the opposite ones (180^o and 270^o, respectively). The moving bar had 4^o width and $25^o/s$ speed, with the dark at $0WHAT$, the light at $204WHAT$ and the background at $102WHAT$. This patch appeared in any of 80 positions in the monitor, which was divided in a 10 by 10 grid, at the 50^o maximum radius from the center. The presentation was repeated in each grid position 14 times, to a total of 1120 trials, at shuffled order whithin each repetition.

At each trial of $1s$, the stimulus played for $220ms$ and was followed by an $880ms$ ITI, summing 19 minutes of RF mapping at each session.

[GET IMAGE]

5.5.2 Tunings mapping stimuli

The selectivity of each neuron was also controlled for spatial and temporal frequencies, as well as for more gratings' directions of movement.

With the same contrast configurations as for the previous protocol, a circular centered patch of 30º was presented at any of 8 directions: the previous and the intermediatedly oriented ones (0º, 45º, 90º, 135º, 180º, 225º, 270º and 315º). The gratings could have 0.02/º or 0.04/º of spatial frequency and $0.5Hz$ or $1Hz$ as temporal frequency. Together, each of these 32 configurations of direction and frequencies were presented in 25 repetitions, totalled at 800 trials and shuffled within the full protocol.

Each trial had $1s$, divided in a baseline of $5ms$, a stimulus presentation of $900ms$ and an ITI of $95ms$, to a total of 14 minutes per session.

5.5.3 Surround Modulation stimuli

Finally, for the SM protocol the frequencies of 0.04/º and $1s$ were chosen based on previous reports of largest V1 stimulation. The light contrasts of the gratings went from 0 at dark to 122.5 at background and 255 at the lightest. Each trial had $2s$, with $0.5s$ of baseline, $1s$ of stimuli display and $0.5s$ of ITI.

There were 124 possible trial types, repeated 20 times, to a total of 2480 trials in an 1 hour and 23 minutes session.

There were 5 possible patches: a central one and four surround patches in the cardinal positions. The central patch was a circle of 15º radius, as the others were limited by an external circumference of 50º, an inner circumference of 27º (to obtain a 12º gap between the center and the surround patches) and the curresponding bissectors of the screen. For any patch, there were 4 available directions of gratings movement (0º, 90º, 180º and 270º).

With these patches, five groups of stimuli types were used:

- C - only the center patch, in any of the 4 directions of movement (4 types);

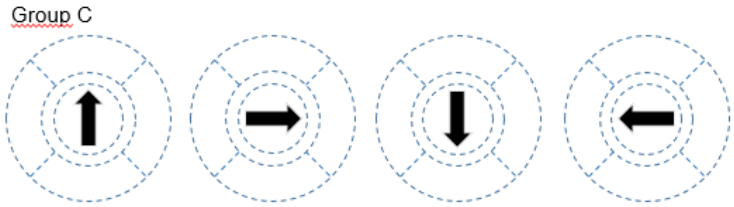

Figure 5.1: Diagram of stimuli group C.

- $S1$ - only one surround patch, in any of the 4 cardinal top, bottom, left or right locations (S1T, S1B, S1L, S1R), and in any of the 4 directions (16 types);
- $S1 + C$ - One surround patch and the center patch, at any location of the surround($S1T + C$, $S1B + C$, $S1L + C$, $S1R + C$) and any direction for the center and for the surround (64 types);

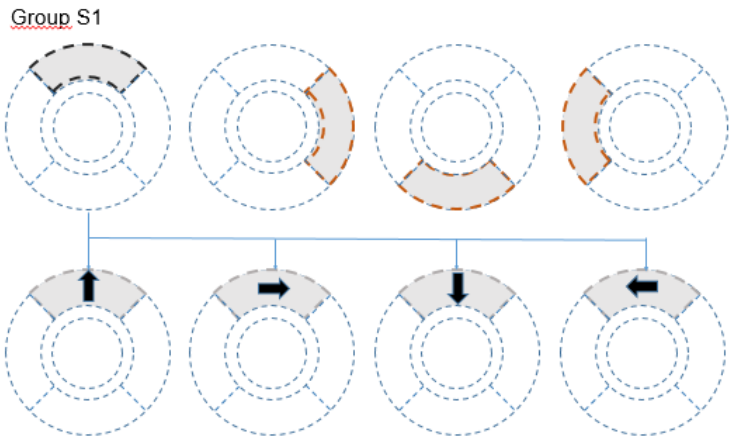

Figure 5.2: Diagram of stimuli group S1.

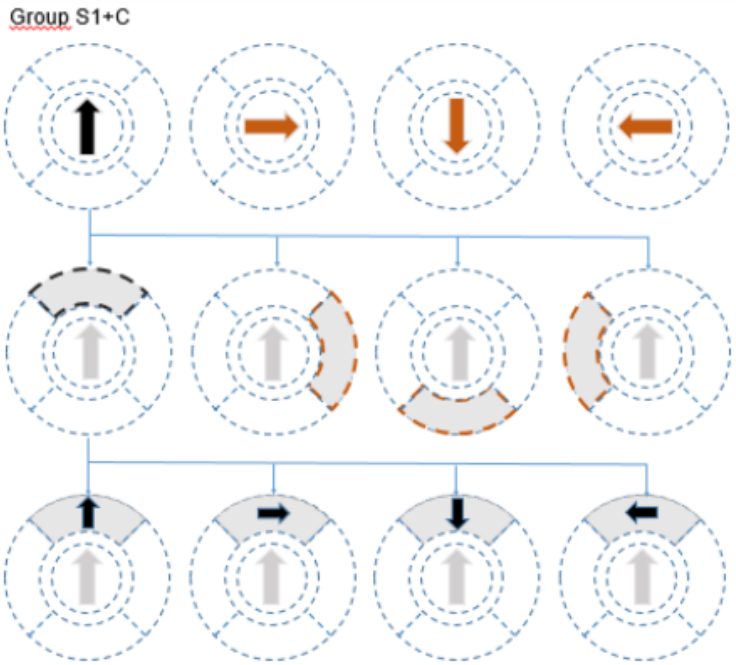

Figure 5.3: Diagram of stimuli group S1C.

- $S2$ - only two surround patches, in opposite cardinal locations, either in the horizontal line ($S2H$) or the vertical line ($S2V$), both of them with gratings moving in the same of any of the 4 directions (8 types);

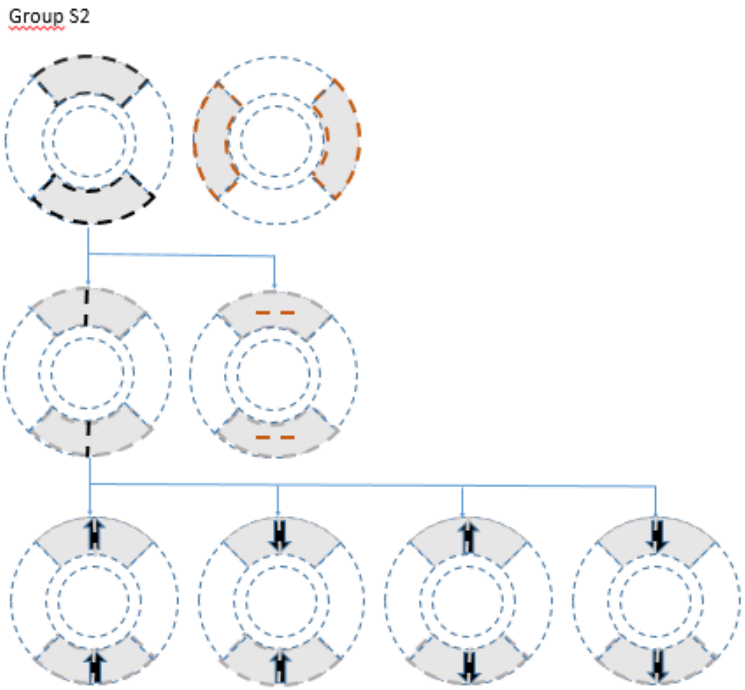

Figure 5.4: Diagram of stimuli group S2.

- $S2 + C$ - Two surround patches and the center patch, both surround patches either in the horizontal ($S2H + C$) or the vertical line ($S2V + C$), both with gratings moving in the same of the possible directions and the center patch with gratings moving in any direction, not necessarily being the same from the surround stimuli (32 types).

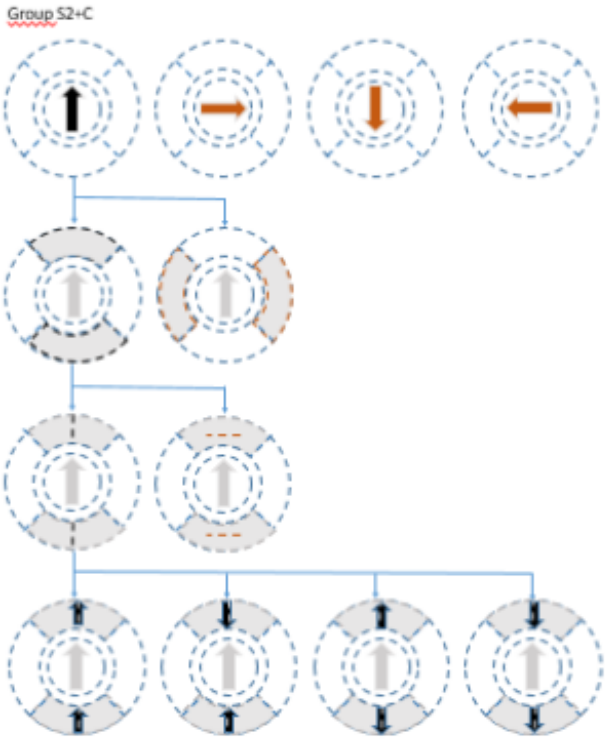

Figure 5.5: Diagram of stimuli group S2C.

Group C was used as a more specific confirmation for the receptive field findings: here the stimuli was now in the center of the screen with the selected size for the analysis. Groups $S1$ and $S2$ provided the data that allowed to exclude from the analysis the cells that responded to stimulation in the defined surround. These had a receptive field that overlapped what we regarded as the surround and thus did not meet the criteria for investigating surround modulation effects in this experiment's designed manner. With the cells that did respond to group C but did not to group $S1$ nor $S2$, we could then regard the effects of actual surround stimulation by examinating the responses to stimuli in the groups $S1 + C$ and $S2 + C$.

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Conclusions Chapter

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