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Organisation rétinotopique des structures visuelles révélée par imagerie optique cérébrale chez le rat normal.

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Ce mémoire intitulé :
Organisation rétinotopique des structures visuelles révélée par imagerie optique cérébrale chez le rat normal.

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RÉSUMÉ FRANÇAIS

Introduction : Il existe dans la plupart des structures visuelles une représentation topographique du champ visuel. L'imagerie optique des signaux intrinsèques des neurones corticaux représente une technique de pointe permettant de visualiser rapidement l'organisation visuotopique avec une excellente résolution spatiale. Cette technique repose sur la mesure des changements des propriétés optiques des tissus en réponse à l'activation neuronale qui ne représente que 0.1% des changements d'absorption par rapport au bruit vasculaire périodique du cœur (5 Hz), de la respiration (1 Hz) et des phénomènes vasomoteurs (0.1 Hz). L'approche la plus couramment utilisée pour éliminer ces bruits consiste à moyenner les réponses ce qui implique de très longues sessions d'enregistrement (>1h).

Matériel et méthodes : Pour résoudre ces problèmes une nouvelle approche se basant sur l'analyse temporelle du signal suite à une stimulation périodique a été développée. Sur le cortex visuel et colliculus supérieur du rat 300-350g (albinos et pigmenté) anesthésié par uréthane, les réponses ont été enregistrées lors du déplacement périodique d'une barre horizontale ou verticale d'une durée de 2 à 6 s. Pour chaque pixel, la composition spectrale des réponses a été analysée à l'aide de la transformée de Fourier permettant d'obtenir l'amplitude et le délai du signal. L'extraction spécifique du signal permet d'augmenter considérablement la qualité des images tout en réduisant le temps d'enregistrement (< 10min).

Résultats : avec cette technique nous étions capable de tracer Les cartes visuotopique du cortex visuelle ainsi que celle du colliculus supérieur. L'organisation rétinotopique de ces cartes obtenues sont semblables à celles définies par électrophysiologie.

Conclusion : Cette nouvelle approche pourra être utilisée également sur différents modèles physiopathologiques (ex : rétinopathie du prématuré), pour étudier l'impact de ces rétinopathie sur les structures visuelles.

MOTS CLÉS FRANÇAIS

Acuité

Analyse de Fourier

Bruit vasculaire

Collicule Supérieure

Cortex visuelle primaire

Facteur cortical de grossissement

Imagerie optique

Ondes de Mayer

Organisation rétinotopique

Stimulation continue

RÉSUMÉ ANGLAIS

Introduction: Most of the visual structures have a topographic representation of the visual field. The optical brain imaging of intrinsic signals is a powerful technique that allows visualizing the visuotopic organization with high spatial resolution.

The OBI relies on measuring changes in optical properties of neuronal tissue in response to neuronal activation. This change represents only 0.1% of the vascular noise: heart beat (5 Hz), respiration (1 Hz) and vasomotor noise (0.1 Hz). The most commonly technique used to eliminate this noise is to average the neuronal signal which means very long recording sessions (>1h).

Materials and methods: To solve these problems a new approach based on spectral analysis has been developed. On the visual cortex and superior colliculus of an anaesthetized rat 300-350g (albino and pigmented), the neuronal responses were stored during a periodic stimulation of a vertical or horizontal bar with a duration of 2 to 6 s. For each pixel, the signal was analyzed using the Fourier analysis to obtain the time and amplitude. The extraction of specific signal allow us to increase the quality of images while reducing the recording time (>10min)

Results: With this technique we were able to map visuotopic organization of visual cortex and the superior colliculus. The organization retinotopic obtained were similar to those defined by electrophysiology.

Conclusion: This new approach could also be used on different physio-pathological models (ex: retinopathy of prematurity), a way to study the impact of retinopathy on visual structures.

MOTS CLÉS ANGLAIS

Acuity

Continuous stimulation

Cortical magnification factors

Fourier analysis

Mayer waves

Optical imagery

Primary visual cortex

Retinotopic organization

Superior colliculus

Vascular noise

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LISTES DES ABRÉVIATIONS

AL: Anterolateral

AM: Anteromedial

CBV: Cortical Blood Flow

CMF: Cortical Magnification Factor

Hb: Haemoglobin

Hbr: Deoxy-haemoglobin

HRF: Hemodynamic response

LGN: Lateral geniculate nucleus

LL: Laterolateral,

LLA: Laterolateral anterior

LM: Lateromedial

OBI: Optical Brain Imaging

Oc1: Primary visual cortex

Oc2: Secondary visual cortex

PCA: Principal Components Analysis

PM: Posteromedial

SC: Superior Colliculus

V1: Primary Visual Cortex

V2: Secondary Visual Cortex

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On the personal front, my love to my parents ,my brother Mohammed and my sisters Miriam and Qatro Al Nada for encouraging me all through my life. I would like to dedicate this thesis to them.

INTRODUCTION

In all mammalian, light reflected by objects reaches the retina, and then its transformed into electrical signals and transferred to the retinal ganglion cells and then to the sub-cortical area (ex: Lateral Geniculate Nucleus (LGN), and Superior colliculus (SC)) via the optic nerve and optic radiation. LGN delivers its information mainly to the brain in the occipital area which is called primary visual cortex. In turn primary visual cortex sends projection to the superior colliculus and other sub-cortical area (pulvinar, etc) and extrastriate areas (ex: V2)

Visual signals are generally processed in two pathways: the dorsal and the ventral. The dorsal pathway starts in the retina with ganglion cells of M type which send its projection to the magnocellular layers of LGN, then reach the layer 4 in the V1. From V1, information is conveyed in the dorsal area of brain, the middle temporal area extending to the posterior parietal cortex. The ventral pathway starts with P cells and via parvocellular layers in LGN projects to 4Cβ and 4A layer of V1, then V1 send their projection to V4 linked with inferior temporal cortex.

The parvocellular (P) fibers transfer colour and high contrast and black and white detail information, in other hand, Magnocellular fibers (M) transfer motion information and low contrast. The ventral pathway is involved in object recognition, whereas the dorsal pathway carries out spatial localization of stimuli and visual guidance of motor actions. It has benn suggested that dorsal pathway is concerned with "where" objects are, and ventral pathway with "what" the objects are (Mishkin *et al.*, 1983).

The visual cortical areas have a hierarchy organization that reflects distinct arrangements of axonal connexion. This organization is the result of a developmental process during which retinal ganglion cell's axons make a series of path-finding choices to reach their targets to establish retinotopic organization. A visual cortical area is considered to be retinotopically organized if nearby locations within this area represent nearby locations in visual space. In primates, the striate cortex (V1), extrastriate visual cortical areas (ex:V2) and sub-cortical areas (ex: SC, LGN) are retinotopically organized,

This cortical retinotopic organization has been studied extensively using a variety of experimental approaches, including lesions studies (e.g., Holmes 1945; Horton and Hoyt 1991), electrophysiological recordings (e.g., Hubel and Wiesel 1974; Talbot and Marshall 1941; Van Essen and Zeki 1978; Van Essen et al. 1984), 2-deoxyglucose autoradiography (Tootell et al. 1982, 1988), cytochrome oxidase staining of the representation of angioscotomas (Adams and Horton 2003), electrical stimulation (e.g., Bradley et al. 2005), positron emission tomography (Fox et al. 1987), functional magnetic resonance imaging (fMRI) (e.g., Brewer et al. 2002; Fize et al. 2003; Larsson and Heeger 2006; Sereno et al. 1995; Wandell et al. 2005).

In recent years, optical brain imaging (OBI) was used to reveal, with high precision, the functional organization of the cortex in various species such as ferrets, cats and primates. Surprisingly, very few studies were carried out in rats, an animal extensively used in models of eye diseases, such as glaucoma. This may likely come

from the fact that intrinsic optical imaging in rats represents a great challenge due to the presence of noise, especially large vasomotor waves (Mayer Waves).

1. The rat visual system

Visual system is a part of the nervous system which permits to analyse and build a representation of the surrounding world. Light reflected by objects reaches the retina where two different photoreceptors classes are located: rods which are sensitive to light's intensity and cones sensitive to the wave length of the afferent light. In order to reach the photoreceptors light must go through other layers in the retina; ganglion cell, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer and outer segment layer. Visual information is transformed into nervous signals. In turn, the signal is transferred to the brain's visual structures via the optic nerve and optic radiations and each type of information will go through a different route to perception. In the rat, most of the axons in the optic nerve go to the superior colliculus.

Figure 1 shows retinal ganglion cells projection from the right eye into many visual structures in the rat brain (i.e. pulvinar, lateral geniculate, superior colliculus, dorsal raphe nucleus, etc.). For simplicity each projection is shown as a single branch of single fibre and the contralateral projections (thick line) are more extensive than ipsilateral projections (think line). However, in all mammalian, lateral geniculate nucleus (LGN) and/or superior colliculus (SC) receive the majority of these projections. In rodents (i.e. mice, rat) most of the retinal ganglion cells project to the SC, making the SC an important structure for studying the effects of retinal degeneration on sub-cortical structures.

1.1 Visual cortex

1.1.1 Anatomy

Like other higher mammalians, the rat visual cortex is positioned in the occipital cortex and has several visuotopically organized areas (Montero et al., 1973a, 1973b). The largest of these areas is the primary visual cortex, also called area 17 or Oc1 (Krieg, 1946a, 1946b). Montero et al., have shown that The primary visual cortex receive a massive projections from the superior colliculus and send projections to other cortical visual areas like: secondary visual cortex

Using immuno-histological technique, Espinoza et al., have found that the surface of the primary visual cortex varies from about 7.1 mm^2 (Espinoza and Thomas, 1983) to about 9.4mm^2 (Peters et al., 1985), constituting 2/3 of the area of all visual cortical regions and about 10-12% of the entire cerebral cortex (Espinoza and Thomas, 1983).

The primary visual cortex of rat consists of six alternative layers. Layer 1 is composed mostly of dendritic and axonal connections. Layers 2 and 3, contain excitatory projection neurons that send projections to extrastriate cortex areas such as area 18a and 18b. Layer 4 receives projections from LGN. And finally, layers 5 and 6 contain excitatory neurons and send most of their projections to the SC.

Many anatomical studies based on retinal lesions (Laties and Sprague, 1966; Wilson and Toyne, 1970; Harting and Guillery, 1976) and electrophysiological studies

(Apter et al., 1945, Siminoff et al., 1966; Feldon et al., 1970; Kruger et al., 1970; Lane et al., 1971; Woolsey et al., 1971; Rhoades et al., 1980) have shown that there is a topographic organization of the primary visual cortex in rat, in which the superior visual field (lower retina) is represented caudally and the nasal visual field (temporal retina) is represented laterally. This organization is referred to as a retinotopic or visuotopic map (Adams and Forrester, 1968; Espinoza and Thomas, 1983; Montero et al., 1973)

The retinotopy map of the rat's primary visual cortex shown in Figure 2 (Adams and Forrester 1964) was obtained by using microelectrode penetrations through the entire primary visual cortex. However this organization was determined by the sum of data from 8 experiments. For more detail see the figure 2

Electrophysiology studies also showed that the rat visual cortex has at least seven other visuotopically organized areas (e.g.: (Oc2L (18a), Oc2M (18b)) (Montero et al., 1973, Espinoza and Thomas, 1987) (Figure 3). These extrastriate visual cortex areas are connected reciprocally with the primary visual cortex (Coogan and Burkhalter, 1990; Montero et al., 1973; Olavarria and Montero, 1981). Figure 4 shows that the primary visual cortex (Oc1) project to both Oc2L and Oc2M, and these projections are reciprocal. Moreover the area LM within the area Oc2L is also connected reciprocally with all areas in Oc2M. The extrastriate cortical areas receive also projections from thalamic structures like: LP, Posterior nuclear complex, and DLG (Schober et al., 1981, Olavarria et al., 1979, Sanderson et al., 1991).

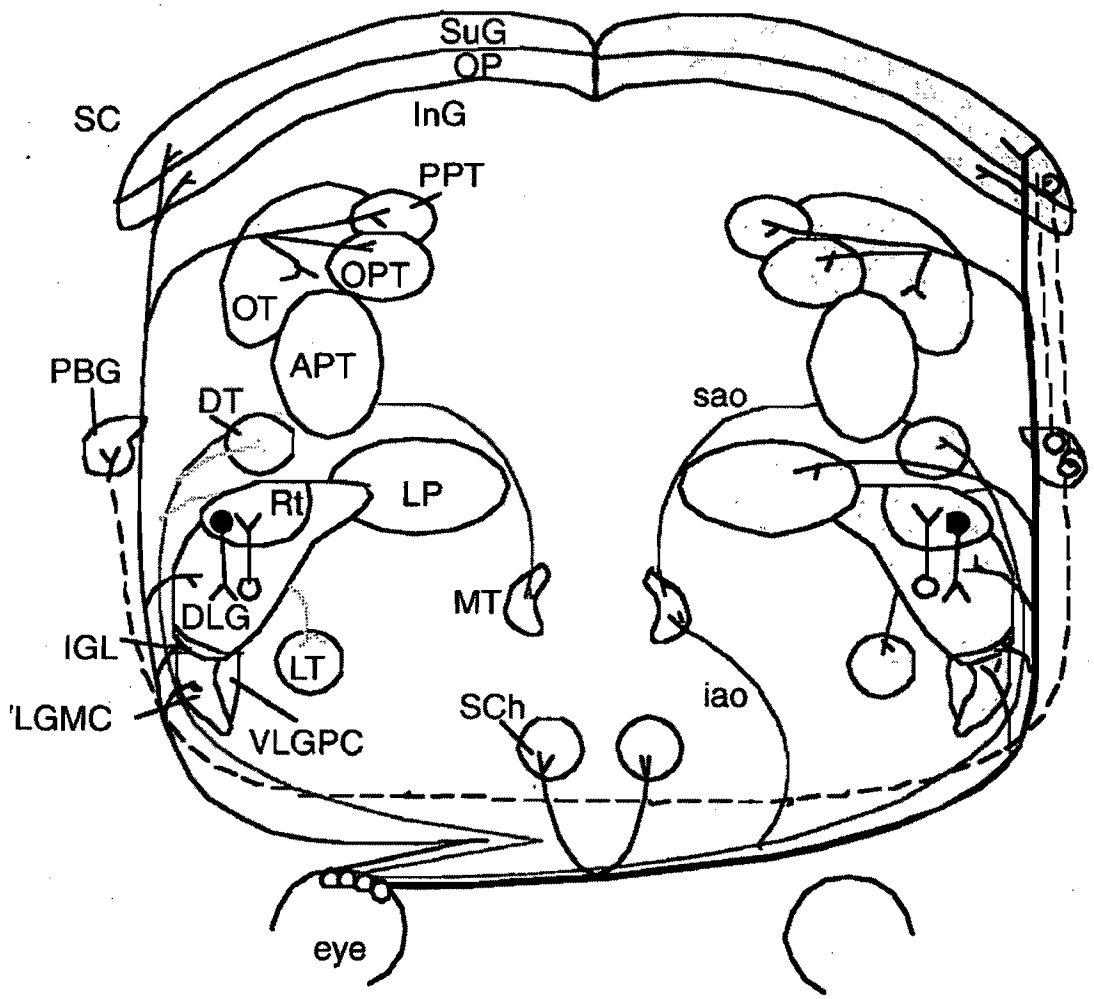

Figure 1.Schematic representation of the retinal projections to the rat's sub-cortical structures. For simplicity each projection is shown as branch of single fibre. Contralateral projections (thick line) are more extensive than ipsilateral projection (thin line). Visual nuclei are labelled: Superior colliculus (SC), lateral geniculate nucleus (LGN), lateral nucleus (LP). From: The Rat Nervous System by George Paxinos, Third Edition (2004).

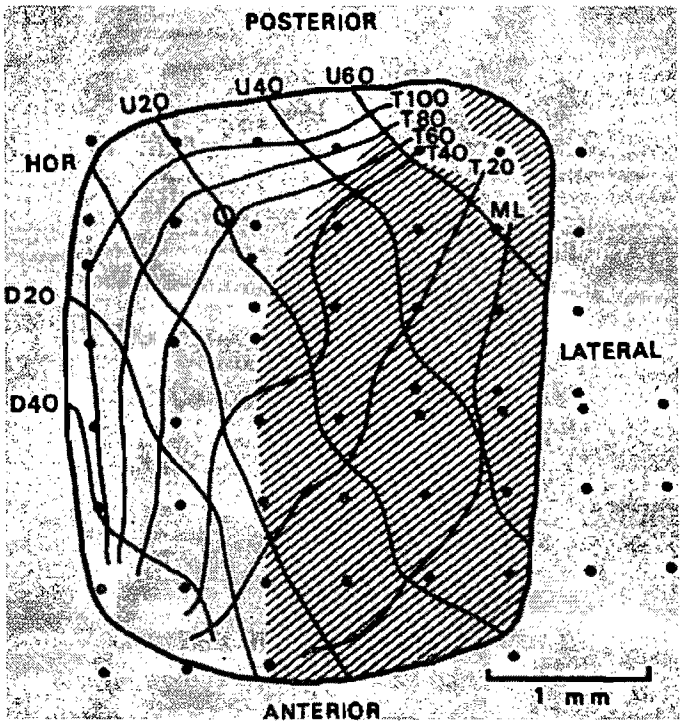

Figure 2: The projection of the visual field on the primary visual cortex of a rat, seen obliquely from the lateral side at 30 degrees to the vertical. ML: meridian in the sagittal plane of the rat. T20: a meridian 20 degrees temporal to ML. HOR: the horizon. U20: a parallel 20 degree above the horizon. D20: a parallel 20 degree below the horizon. 0: position of the optic disc. •: electrode positions. The binocular area, to which the left eye projects as well as the right, is shaded. From: Adams and Forrester (1964)

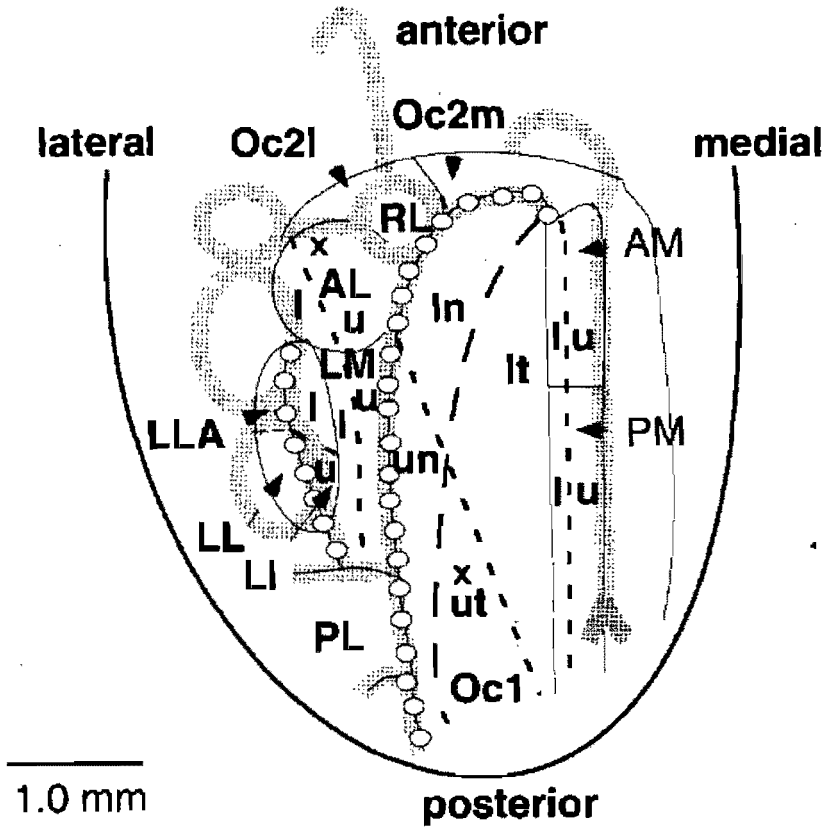

Figure 3. Representation of the visual field of the right eye on to the left visual cortical areas. Striate and extrastriate areas are visuotopically organized. Oc1: Primary visual cortex. Oc2l and Oc2m are secondary visual cortex 18a and 18b respectively. Subdivision AM (anteromedial) and PM (posteromedial) are within Oc2M; AL (anterolateral), LL (laterolateral), LLA (laterolateral anterior), LM (lateromedial) are within Oc2L. Each subdivision contains a representation of the visual field. ut: upper temporal, un: upper nasal, lt: lower temporal, ln: lower nasal. From: The Rat Nervous System by George Paxinos, Third Edition (2004).

1.1.2 Neuron properties

Studying the properties of rat primary visual cortex cells is difficult because the rat has low acuity and the visual cortex is not well developed like other mammalians. However, there are some studies that have been done in electrophysiology to investigate the properties of receptive field neurons in the primary visual cortex of the rat. These studies identified two main groups of neurons that respond differently. The first group of cells responded only to flashing on-off stimulus, and second group responded best to moving sine wave gratings, generated by a computer screen, of an appropriate spatial frequency, but did not respond to ON and OFF stimulus. (Burne et al., 1984; Parnavelas et al., 1981, 1983; Shaw et al., 1975), within these cells, around 2/3 showed a preference for grating oriented horizontally, and 1/3 showed no preference to orientation (Montero et al., 1981; Parnavelas et al., 1981; Fagiolini et al., 1994). This aspect of horizontal preference has been investigated in this study.

2.2 Superior Colliculus

2.2.1. Anatomy and organization of the SC in rat

Like other mammalians, The SC in rats appears as a large protrusion on the midbrain and is made up of many layers. According to numerous studies, the horizontal laminated organization of the SC is organized into seven layers: zonal, superficial gray, optic, intermediate gray, intermediate white, deep gray, and deep

white (Huber and Crosby, 1943). The superficial gray and upper optic strata are innervated by retinal ganglion cell axons that transmit visual inputs to SC with magnification factor of 45mm/degree along the anteroposterior axis and 24mm/degree along the mediolateral axis (Siminoff et al 1966). Cells in superficial layer project to intermediate and deep layers, which also receive inputs from other sensory systems like: LP, DLG (Harting et al., 1973; Stein and Gaither, 1983).

The retinal inputs to the rat's SC cross over almost completely, however 98% of retinal ganglion cells in pigmented rats and 95% in albino rats' project to the contralateral hemisphere (Lund, 1965). The right hemisphere of the SC contains a topographic representation of the left visual field (derived from input from the left eye) and the left hemisphere contains a topographic representation of the right visual field (inputs from the right eye). Because this crossover of afferents is not complete, there are few ipsilateral retina inputs apparent (2% in pigmented rats and 5% in albino rats) (Siminoff et al., 1996). These ipsilateral projections result in a binocular zone in the rostral part of the SC (Tiao & Blakemore, 1976).

The rat visual field is about +20 degree to +120 degree in azimuth axis and -40 degree to +40 degree in elevation axis, and rat's SC has a large receptive filed between 3 degree to 15 degree and the size expands from superficial to deeper layers. Most of the receptive field of SC cells in superficial layers have two spatial organizations: a uniform (ON, or OFF) or concentric (ON/OFF). However some cell showed a preference to direction selectivity to light spot moving at high velocity (Siminoff et al 1966). The spatial frequency of SC cells is very low (0.1

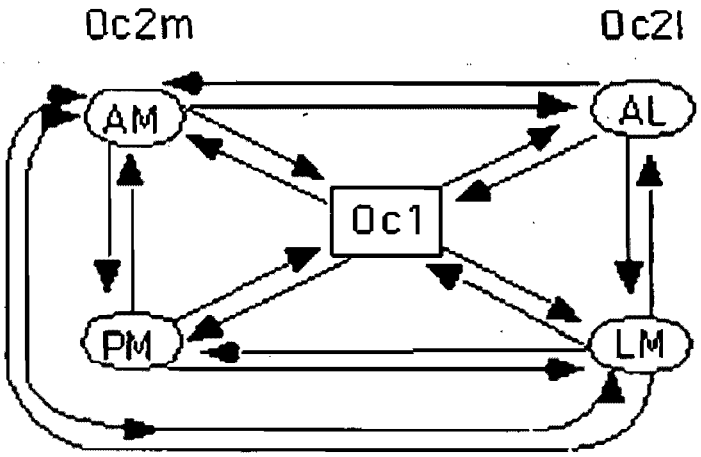

Figure 4. Schemas of interconnections between striate and extrastriate visual cortex viewed as if from above. Reciprocal connections are indicated with double headed arrows. . Oc1: Primary visual cortex. Oc2l and Oc2m are secondary visual cortex 18a and 18b respectively. Subdivision AM (anteromedial) and PM (posteromedial) are within Oc2M; AL (anterolateral), LL (laterolateral), LLA (laterolateral anterior), LM (lateromedial) are within Oc2L. From: The Rat Nervous System by George Paxinos, Third Edition (2004)

cycle/degree) compared to the rat's primary visual cortex (0.6 cycle /degree) or to SC cells in the cat (2.2 cycle per degree)

The SC receives afferent projection from the striate and extrastriate visual cortex. The corticotectal projections originate in layer five of the primary visual cortex (Hubener and Bolz, 1988) and terminate at different layers within the SC. Many studies have investigated the role of cortico-tectal projections. Most of these studies suggest that the activity of the superior colliculus in the rat is controlled by two opposing influences: corticotectal facilitation and tectotectal inhibition (Goodale et al., 1973).

Goodale et al., have shown that destruction of one superior colliculus and recording in the remaining contralateral superior colliculus showed an increase in the amplitude of the light-evoked potential (Goodale et al., 1973). However unilateral ablation of the primary visual cortex by aspiration or cooling produced a depression of the light-evoked potential recorded in the ipsilateral superior colliculus (Goodale et al., 1973).

Sub-cortical structures such as the SC also have orderly organizations of the visual field (Figure 5). Cells with receptive fields in nasal visual space are located rostral, whereas cells with receptive fields in temporal visual space are located caudal. Cells with receptive fields in the upper visual field are located medially and those with receptive fields in the lower visual field are located laterally.

2.2.2 The role of the SC in vision

The superior colliculus plays an significant role in spatial localization of objects, its role is *identifying where the object is rather than what it is.* The functional role of the SC includes visual attention and orientation to an objet (Goodale et al., 1978; Sahizada et al., 1986), head movement (McHaffie and Stein, 1982; Okada, 1992), and escape danger (Olds, et al., 1962). These roles of the SC are attributed to the deeper layers. It has been shown that damage in the SC causes visual and sensory neglect (Krauthamer et al., 1992) and deficit in visual search (Heywood and Cowey, 1987).

To understand the role of the superior colliculus and other visual structure a new method was developed in the last decades, called optical brain imaging (OBI) of intrinsic signals, which has been successfully used to reveal with high precision, the functional organizations of the cortex in various species such as mice (Schuett et al., 2002; Kalatsky and Stryker, 2003), rats (Gias et al., 2003, 2007), ferrets (Li et al., 2006), cats (Swindale et al, 2003) and monkeys (McLoughlin et al., 2005). Recently, this technique has been used to determine the organization of the superior colliculus in the mouse (Mrsic-Flogel et al, 2005).

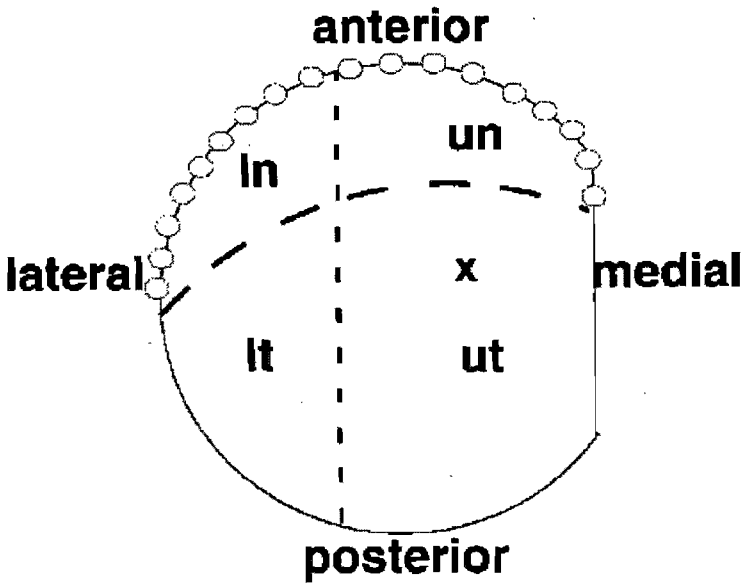

Figure 5. Schematic diagram of representation of the visual field of the right eye on superior colliculus. Each subdivision contains a representation of the visual field. ut: upper temporal, un: upper nasal, lt: lower temporal, ln: lower nasal. From: The Rat Nervous System by George Paxinos, Third Edition (2004).

2. Optical Imaging technique

2.1 Introduction

Optical Brain Imaging of intrinsic signals (OBI), developed by Grinvald and co-workers (Bonhoeffer and Grinvald,1996; Frostig et al., 1990; Grinvald et al., 1986, 1999; Ts'o et al., 1990) has been widely used to reveal the functional organization of the normal brain in both humans and animals. OBI allows the investigation of the functional architectures of the brain and to study the plasticity of cortical areas under different experimental manipulations and in experimental models of neuropathology.

In OBI, the intrinsic signal measures the variation of the intrinsic changes in the optical properties of an active brain zone. This variation has different components which originate from different sources. The variation in these components derives from the fact that neurons activity is related to local metabolic activity and blood component variation. There are three main components (Bonhoeffer and Grinvald 1992):

1- Cortical Blood volume changes (CBV).

2- Blood oxygenation changes (Deoxy-haemoglobin and haemoglobin).

3- Light scattering changes caused by ion and water movement.

In activated cortical area (Ex: Visual cortex, superior colliculus) there is an initial increase in the concentration of deoxy-haemoglobin (decrease of oxy-haemoglobin) due to the increased oxygen consumption by the cells, followed by an increase in highly oxygenated cortical blood flow (CBV). As a result of these changes, the activated neurons zone reflect less light for given wavelength and the darker regions of the optical imaging maps represent the activated areas of the cortex.

Each component of the intrinsic signals has different time-course. To detect a component of interest, one must illuminate the cortical area with the proper wavelength (Blood et al., 1995; Frostig et al., 1990):

1- Green Light, 546 nm: This wavelength was used to obtain anatomical image (blood vessel surface).

2- Orange Light, 605 nm: This wavelength detects the changes of the oxymetry component.

3- Red light, 630 nm: At this wavelength the intrinsic signal is a mix of the blood volume and desoxy-hemoglobin/ haemoglobin. This wavelength was used in this study

4- Near infrared filters, 750nm-800nm: At this wavelength range, the intrinsic signal is dominated by the light scattering component

The choice of wavelength is still under debate and it has been shown that functional maps obtained at different wavelengths are almost identical with small variation on the ration signal/noise. Nonetheless, all of these wavelengths can be

used for functional mapping. Further explanation concerning the choice of wavelength can be found in the "discussion" section.

In the last two decades optical imaging studies have investigated numerous cortical functional architecture that were first discovered by Hubel and Wiesel (Hubel and Wiesel 1962), such as ocular dominance in the primary visual cortex in monkeys (Ts'o et al., 1990) and cats (Swindale et al., 2003), or organization of orientation preference (Grinvald, et al., 1986; Bonhoeffer et al., 1993).

Figure 6 illustrates an example of orientation map columns in area 18 of cat using continuous stimulation. The stimulus was sine wave grating projected on big screen that change orientation continuously. The amplitude map represents the strength of the optical signals and the color code in the phase map represents the specific orientation of each stimulus. The sum of these two maps is found in the orientation column map. Circle on the center represent the location of pinwheel

With the advancement of this technique it was possible to explore more delicate cortical organization, such as direction selective columns or spatial frequency columns (Malonek et al., 1994; Shmuel and Grinvald, 1996; Weliky et al., 1996).

OBI of intrinsic signals has also allowed us to see the color selectivity neuron in V1 (Landisman and Ts'o, 2002) and functional architecture of others areas like area MT in monkeys and area 21 in cats (Villeneuve & Casanova 2008: Submited).

Recently, many studies have mapped the retinotopic organization of rodents such as mice (Kalatsky and Stryker 2003) and rats (Gias et al., 2003, 2007). They

confirmed that these two species have an orderly cortical arrangement of the visual field but there was no evidence of orientation column maps. Figure 7 shows some work done in husson et al., 2007, to map the retinotopic organization of the primary visual cortex in mouse using continuous stimulation in. Each color code in the figure represents a specific location of the stimulus on the visual field.

Others studies were done on auditory cortex (Harrison et al., 1998; Harel et al., 2000) and somato-sensory cortex of rodents (Blood et al., 1995; Brett-Green et al., 2001). In agreement with electrophysiology studies, these studies found a low to high frequency tonotopic organization,

Optical imaging allows the investigation of the functional maps in pathological models and developmental studies such as deactivation of cortical areas, monocular deprivation, glaucoma models, and retinopathy models. Optical imaging has examined the plasticity of visual cortical maps in response to many manipulations of the visual inputs including strabismus (Engelmann et al., 2002), monocular and binocular deprivation (Crair et al., 1997, 1998).

All of these studies have generally demonstrated that the size of individual functional domains as well as retinotopic maps can be affected (e.g. map distortion, size of orientation and/or direction columns). The next section will discussed the origin of these maps in greater detail

Orientation Amplitude Orientation column map

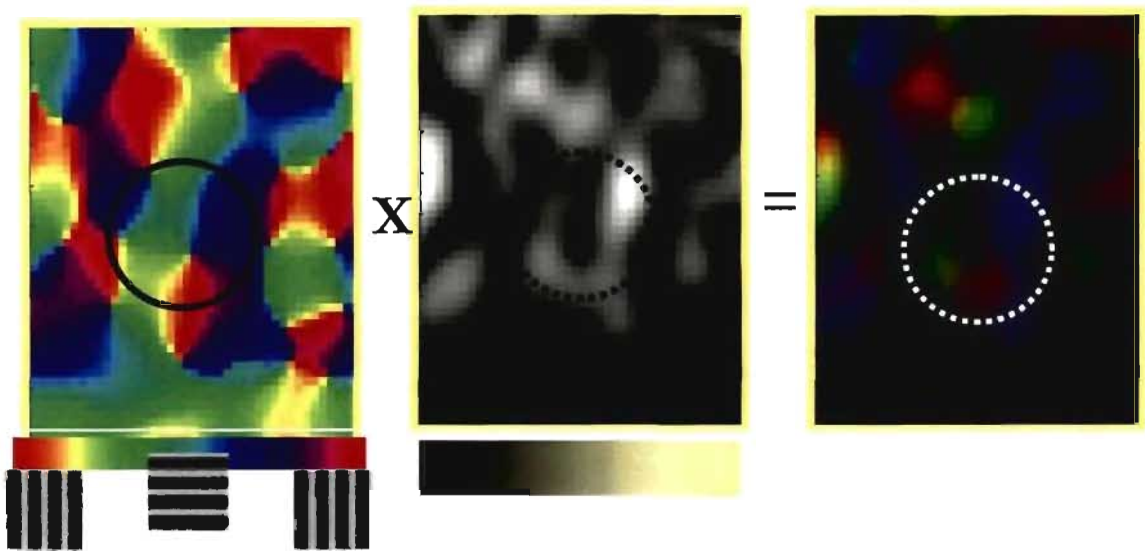

Figure 6: Orientation map of area 18 in the cat using continuous stimulation. Circle represents pinwheel. Each color in the map represents specific orientation of the stimulus. Gray scale represent the strength of the signal. Figures from work done by Mathieu Vanni

2.2 Cortical maps

The brain of most mammals contains orderly maps of diverse sensory and motor inputs. Auditory cortex contains a map of sound frequency, somato-sensory cortex contains a map of the body surface, visual cortex contains a retinotopic map of visual space and motor cortex contains a map of the body musculature. Within the same sensory cortex we can find different types of maps. For example, in the visual cortex of higher mammals (cat, monkey, rabbit, etc) we can detect orientation, ocular dominance, retinotopy, and spatial frequency maps.

The question that we must ask is why do such maps occur? It would be foolish to think that retinotopy maps are derived solely from the fact that axons from adjacent retinal ganglion cells project to adjacent location in the primary visual cortex. However, many experiments in cats and rats (Horton et al. 1979, Simon & O'Leary 1991) have prove that neighbouring retinal ganglion cells are scattered in the optic nerve but they still give rise to visuotopic maps on LGN, SC and primary visual cortex, this statement confirm that there are others intrinsic factors responsible for the formation these maps.

Many studies have shown that the formation of these maps depend on sensory inputs during development phase called "critical period" (Wiesel and Hubel, 1963; Blakemore and Cooper, 1970) in which two neurones get together when pre-synaptic and postsynaptic neurons are active simultaneously. This occurrence is

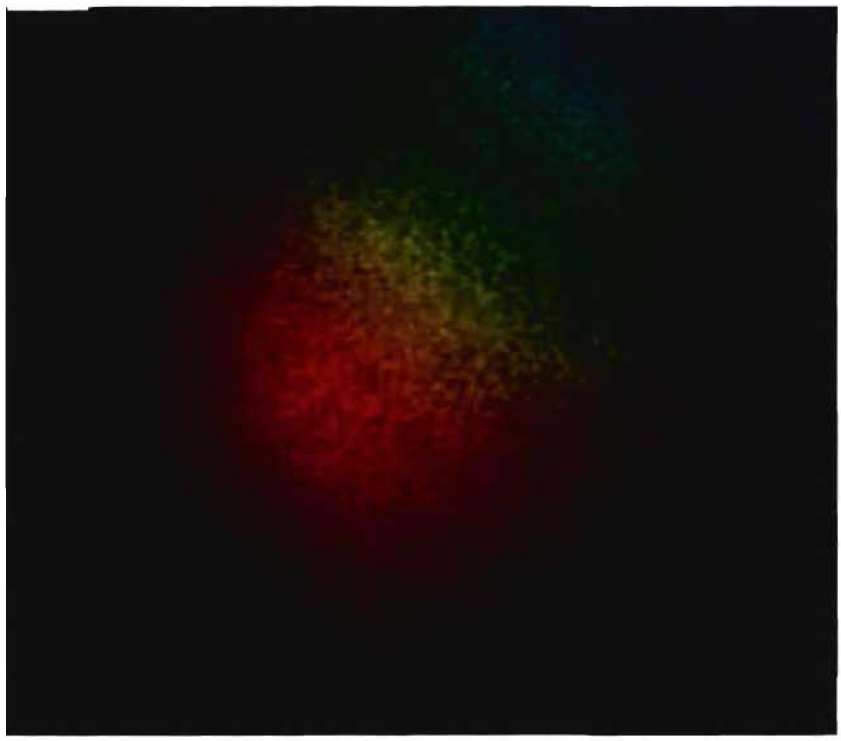

Figure 7: Retinotopy map in primary visual cortex of mouse using continuous stimulation. (drifting horizontal bar) Each color code represents a specific location of stimulus on the visual field (Husson et al., 2007)

called the Hebbians Rule and states that "cells that fire together get together." This phenomenon may be explained by the wiring optimization principle; in which neurons with close receptive fields should be near to each other, allowing close neurons in the visual field to network over short axonal and dendritic projections, permitting communicate in short time and minimizing the wiring cost (Allman and Kaas 1974)

In 1997, Hübener and co-workers reported that in higher mammalian (i.e. cats and monkeys) the maps of ocular dominance, spatial frequency and orientation tend to superimpose and intersect one another at right angles. Many studies have hypothesized that the organization of these cortical maps may be designed to optimize coverage, in which cortical maps are organized in such a way as to "optimize the efficiency of transmitted information" (Allman & Kaas 1974).

To confirm this hypothesis, Swindale et al., (2003) have generated artificial maps of different stimulus proprieties (spatial frequency, orientation, etc.) with different combinations. Then they compared these artificial maps with real maps using a variety of quantitative parameters. Swindale et al., have found that these cortical maps organizations originate from two constraints:

1-The continuity constraint: adjacent neurons should have the same receptive field properties,

2- The coverage uniformity: the mixture of the parameters represented in each maps must be spread uniformly over visual space." (Swindal et al 2001).

This study concluded that these well designed and interrelation cortical maps are intended necessary to optimize coverage

3. Stimulus and analysis of functional maps

3.1 Conventional methods

The conventional stimulus protocol in OBI is periodic. It consists of presenting many episodic stimuli in different or in the same region of the visual field to trace retinotopy, orientation or direction maps, etc. For example, to trace the retinotopic map, one stimulus is first presented in a specific region of the visual field for many seconds, during which the emitted light across a region of visual cortex is collected, and then followed by a relaxation period for the optical properties to return to a normal state. Next, a second stimulus is presented in another different region of the visual field, and so on, until the entire visual field is covered. Most of these stimuli are waves grating that change orientation simultaneously in order to activate the maximum of cells

The analysis of functional maps can be obtained by averaging many responses to different stimuli with one another, or by subtracting the pre-stimulus frames from the stimulus frame (Analysis approaches will be discussed in more detail in a later section.).

The paradigm of this technique is shown in figure 8. Panel A illustrates the stimulus design on the visual field (sine wave grating). Panel B depicts the color code of the visual field location of the stimulus, in which each stimulus position was given a specific color code. Panel C shows the average response map of activity across mice for each visual field location. The individual retinotopic map is

portrayed in panel D in which each color code represents a specific location of the stimulus on the visual field.

The difficulty with the conventional approach is that there are many fluctuations in the collected data that are not associated to the stimuli. These fluctuations are due to vascular noise like: respiration, heartbeat, or Mayer waves (oscillations of arterial pressure occurring in subjects at a frequency lower than respiration (i.e., in rat 0.1 Hz)). These fluctuations are not periodic, and since only the average responses are stored, it is difficult in some cases to separate the "noise" from the signal of neural activity even after further analysis with Z score analysis, first frame analysis, or principal component analysis. To overcome this issue it was crucial to adapt another optical imaging paradigm in which all frames must be saved.

3.2 Continuous periodic Fourier imaging

stimulation and continuous data acquisition. To map the retinotopy of cortical or sub-cortical areas, this method consists of presenting a white thin bar in a black background sweeping the visual field with specific frequency during a short period of time (e.g. ten minutes). This stimulus paradigm was designed to excite all neurons at consecutive phases of the stimulus and to record not just the average but all frames time series, the advantage behind that is the detection of all signal components separately.

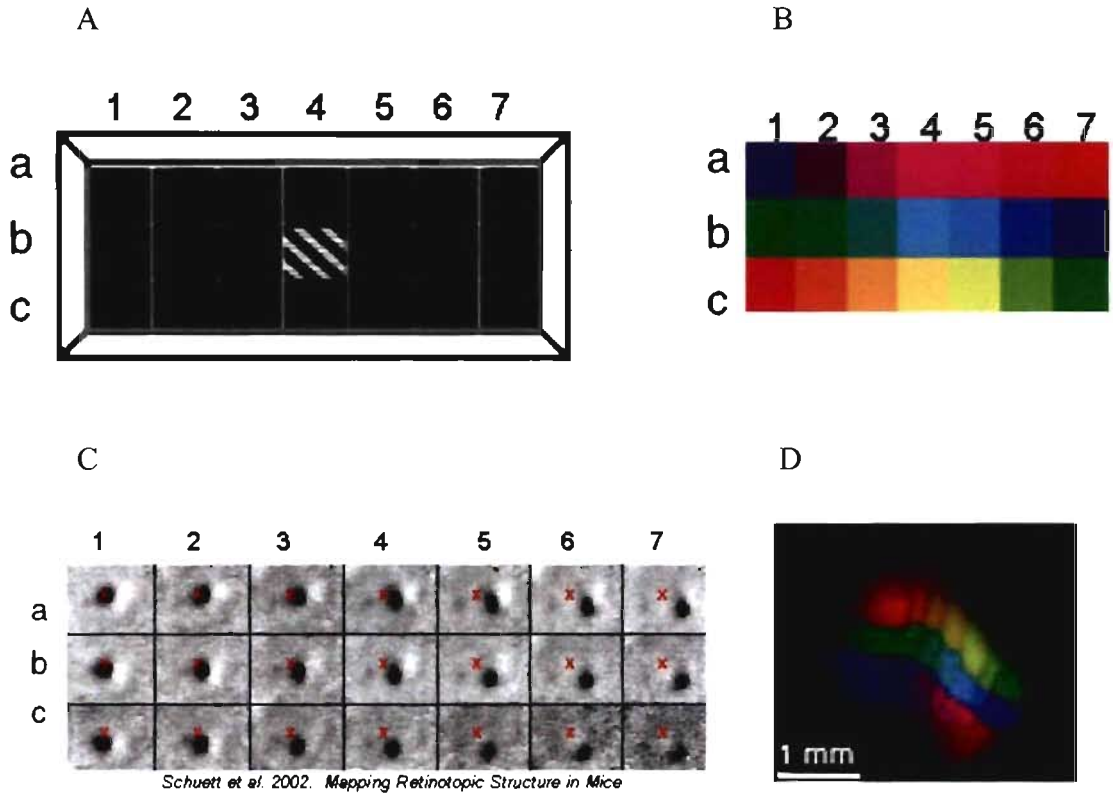

Figure 8: Retinotopic organization of the visual cortex in the mouse using conventional methods in optical imaging. (A): Wave grating Stimulus. (B): Color code of the visual field location of each stimulus (C): Averaged response maps of activity of the visual cortex in mice for each visual field location. (D): Individual mouse retinotopic map. The color code represents the visual field location of each stimulus that produces activation. Red Cross: to indicate that the activated region is moving. From : Schuett et al., (2002).

This new method was first developed for fMRI recording and was later adapted in OBI by Kalatsky and Stryker in 2003, which is based on continuous

To trace the representation of azimuth map, one must used a period vertical bar across the visual field and for the representation of elevation map; one must used a period horizontal bar across the visual field (Figure 9, C, D). Then the color codes were used to visualize the cortical responses evoked by the stimulus, each color represents specific location of the stimulus in the visual field (Figure 9, E, F).

As was mentioned above, the intrinsic signal of optical imaging suffers from many internal dynamic components that contaminated the data. The frequency of the major components of the haemodynamic signal that may contaminate the intrinsic signal of the brain activity are heart beat (2–5Hz), respiration rate (0.3–1 Hz), and vasomotor signal (0.05–0.1 Hz) (Mayhew et al., 1996). If one chooses the stimulation frequency that coincides with these internal components, the response will be dominated with the artefact, and it will be difficult to extract the signal from the artefact and to localise the brain activity. To avoid this artefact, stimulation frequency must be chosen between 0.3Hz and 0.1 Hz.

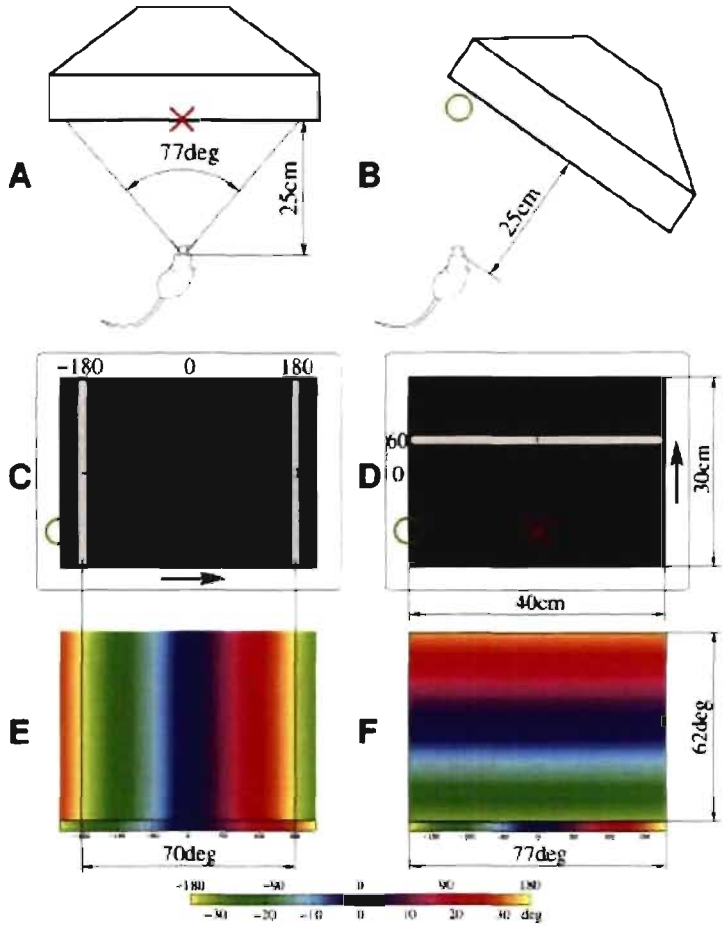

Figure 9: schematic of the stimuli employed to map retinotopy of the mouse visual cortex (A) Setup used to stimulate both ipsilateral and contralateral eyes. (B) Setup used for stimulation of one eye. (C and D) the stimulus patterns: white thin bar sweeping the visual field. (E and F) the color-coding used to visualize the cortical responses evoked by stimuli shown in (C) and (D). The color code diagram represents the visual field. From: Kalastky and stryker (2003).

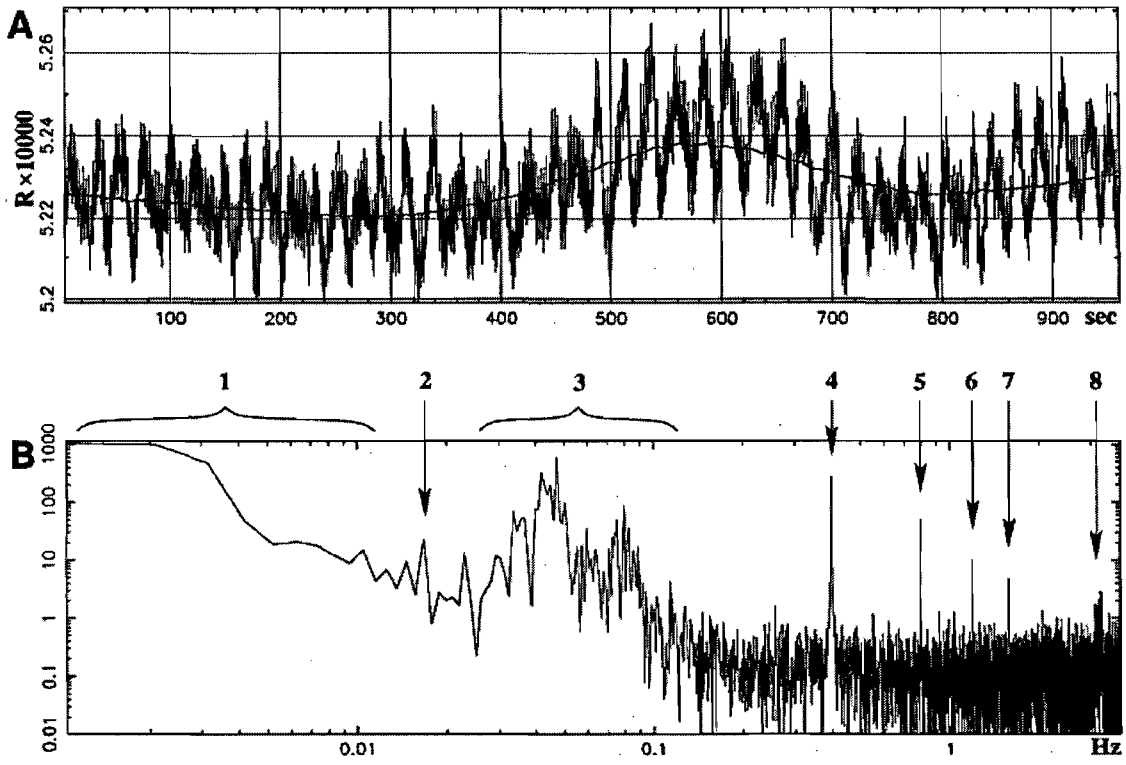

Figure 10. Spectral decomposition of intrinsic signal at single pixel from a series of frame of the cat visual cortex. (A) Time course of light reflectance. (B) The power spectrum of the reflectance signal plotted in (A). (1) slow variation, (2) response at the frequency of stimulation (1 min period), (3) vasomotor signal, (4) ventilation artifact (5), (6), and (7) second, third, and fourth harmonics of the ventilation artifact, and (8) heart beat artifact. From : Kalatsky and Stryker (2003).

To localize the brain activity, Fourier analysis was used to extract neuronal activity at the frequency of stimulation from all frames acquired at each pixel. The Fourier analysis transforms the function time signal into a frequency spectrum. The result of the decomposition is a signal of many harmonics of different frequencies. The formula below (1) represents the spectral decomposition of signal in which, the function "f": is a function of time and represents a physical signal. The Function "F" represents spectrum of the signal in time domaine.

$$F(\omega) = \int f(t)\, e^{i\omega t} dt \qquad (1)$$

Spectral decomposition: f (t) is the spectre. ω: stimulation frequency. F (ω) is the signal after Fourier decomposition.

The spectral decomposition gives information about the phase and magnitude of the spectra, and each peak represents specific components in specific frequency (respiration, heart beat, Mayer wave, etc.), allowing the detection of the neuronal response at the frequency of stimulation we can then construct the functional maps. Fourier analysis will be further addressed in the "materials and methods" section as well as the "discussion" section.

In figure 10, Panel A represents the reflected intensity signal at one pixel in function of time. Panel B depicts the spectral decomposition of the signal at one pixel. Each peak signifies a specific component (heart, Mayer waves, neuronal signal, etc.) for more detail sees the figure 10.

PURPOSE OF THIS STUDY

There were three main goals of this study. The first objective was to build the new paradigm of continuous stimulation and data acquisition in our laboratory because conventional methods based on averaging were not considered suitable for intrinsic optical imaging in rats due to the vasomotor noise and other considerations. Also, developing the new approach is useful for many applications, particularly under conditions where the overall data acquisition time is limited (ie, mapping two visual structures in the same animal).

The second aim was to examine the retinotopic organization of the visual cortex in both albino and pigmented rats, and to compare the magnification in both species. The cortical magnification factor (CMF) is defined as the scaling factor that relates a distance in the visual field (in degrees of visual angle) to the cortical distance (in mm) of the corresponding cortical representation (Daniel and Whitteridge, 1961).

The final aim was to map the superior colliculus of the rat by OBI, a structure that receives massive projections of retinal ganglion cells. However in order to obtain images, the overlying cortex was removed by aspiration.

ARTICLE

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New Tools for Mapping the Rat Visual Cortex and Superior Colliculus by Optical Imaging

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ABSTRACT

Optical brain imaging is a powerful method to characterize the functional organization of visual structures. It has been used in several species to determine the representation of visual space, i.e. the visuotopic organization, in cortical areas. In this study, we investigated the possibility to reveal visuotopic maps in rats using an experimental paradigm based on the continuous stimulation and Fourier decomposition of the signal, recently developed in mice by Kalatsky and Stryker (2003). Experiments were carried out on pigmented and albino rats. Given that optical visual response in rats were rather small in amplitude, we developed a correction method to reduce the noise originating from light fluctuations, which was found to be the major source of noise. This correction considerably increased the signal to noise ratio. Other sources of noise came from respiratory movements and vasomotion. The latter was constituted by two components which peaked at 0.04 and 0.1 Hz and represented a minor cause of noise in both rat types. After correction, visuotopic maps of the primary visual cortex could be acquired in 10 minutes or so. In all animals, the cortical region activated (area V1) was elliptical and visuotopic organizations along the azimuth and elevation were clearly visible. In one case, area V2 was also activated. A new method measuring the cortical magnification factor (CMF) was developed to take advantage of the continuous paradigm. The CMF map was generally homogeneous with a mean factor of 44 μm/deg. Finally, the rat superior colliculus could also be imaged after aspiration of the cortex using the same experimental approach. In conclusion, the combination of light fluctuations correction and continuous stimulation paradigm allows the successful mapping and

quantification of visuotopic maps in animal models largely used for the study of retinal diseases such as glaucoma.

INTRODUCTION

It is well established that visual structures in the brain such as the lateral geniculate nucleus, superior colliculus and visual cortex present an orderly representation of the visual space, namely a visuotopic organization. For the past decades, such an organization has been revealed by neuroanatomical-based studies using circumscribed retinal lesions (Laties and Sprague 1966; Garey and Powell 1968; Wilson and Toyne 1970) and electrophysiological-based studies (Siminoff et al. 1966; Lane et al. 1973; Palmer et al. 1978; Tusa et al. 1978; Tusa et al. 1979; Hutchins and Updyke 1989). In recent years, the complete or partial mapping of visual structures has been used to detect the functional impact of diseases such as glaucoma and macular degeneration (King et al. 2006).

In recent years, optical brain imaging (OBI) of intrinsic signals was successfully used to reveal the functional organization of the cortex in various species such as mice (Schuett et al. 2002; Kalatsky and Stryker 2003; Cang et al. 2005a; Cang et al. 2005b; Cang et al. 2008), rats (Gias et al. 2005; Gias et al. 2007), ferrets (Yu et al. 2005) tree shrew (Bosking et al. 2000) and monkeys (McLoughlin et al. 2005). Recently, this technique has been used to determine the organization of the superior colliculus in the mouse (Cang et al. 2005b; Mrsic-Flogel et al. 2005; Cang et al. 2008). Compared to traditional methods such as electrophysiological

mapping, OBI has the advantage of avoiding long and tedious recording sessions while offering a high spatial resolution and a detailed representation of the visual maps.

Surprisingly, very few studies using OBI were carried out in rats. This may likely come from the fact that OBI in rat represents a greater challenge due to the overwhelming presence of non-periodic (e.g. emitted light instabilities) and periodic (e.g. respiration, heart beat or vasomotion) noises. Vasomotion, also named "Mayer waves" are low frequency oxymetric waves moving along the cortex which strongly reduces the signal to noise ratio (Japundzic et al. 1990; Mayhew et al. 1996). To our knowledge, only one study addressed the visuotopic organization in rats (Gias et al. 2005) using a standard episodic acquisition method. The visuotopic maps obtained with this technical approach have a low definition because a limited number of circumscribed visual stimuli are presented in the visual field. One consequence would be a misevaluation of the limits of the visual structures and of the magnification factor, i.e., the scaling factor that relates a distance in the visual field to that on a given visual area (Daniel and Whitteridge 1961; Pointer 1986), and that could be critical in experiments aimed at mapping visual structures. Moreover, at the practical level, this method necessitates long recording sessions.

A new approach has been recently described by (Kalatsky and Stryker 2003) which is based on the continuous stimulation of the visual field and the Fourier decomposition of the signal. In contrast to the standard episodic method, this periodic paradigm allows the stimulation of all regions of the visual field, thus

increasing the spatial resolution. In addition, the spectral separation of the signal from periodic noise enables to enhance the quality of the maps and to considerably reduce the acquisition period (generally by a factor of 10).

The goal of this study was three-fold. The first aim was to demonstrate that the continuous stimulation paradigm can be successfully used to quantity the visuotopic organization of the visual cortex and superior colliculus (SC) in albino and pigmented rats, two species extensively used in eye disease models, The second aim was to document the contribution of noise in the signal and develop a method for reducing non periodic fluctuations caused by light instabilities. The third aim was to take advantage of the high resolution of the maps and present a new method for measuring the magnification factor for each pixel. Parts of these data have been presented in abstract form (Nassim et al. 2007).

MATERIAL AND METHODS

Animal preparation

Experiments were carried out in Long Evans (n=5) and Sprague Dawley rats (n= 5) weighing 250-300g. All procedures were done in accordance to the guidelines of the Canadian Council for the Protection of Animals and the Ethics committee on the use of animals of the Université de Montréal. Rats were anaesthetized with urethane (1.25 g/kg ip). The level of anesthesia was monitored throughout the experiment (i.e., using a leg stretch reflex) and supplemental doses of anesthetic were

administered when needed. The rats were first placed in a stereotaxic apparatus using mouth and ear bars. The temperature was maintained at 37.0°C by a feed-back controlled thermometer. Electrocardiogram (ECG) was continuously monitored. A unilateral craniotomy was performed anterior to lambda to expose the visual cortex; the dura mater was left intact. A plastic chamber (in house design; 8 mm in diameter and 2 mm height) was placed over the craniotomy and attached to the skull with dental cement. The chamber was filled with 2% agarose and a glass cover slip was secured to its surface. Atropine sulfate (1%) was used to dilate the pupils and the cornea was protected from desiccation by applying artificial tears (carboxyl methylcellulose). The eyes were fixed with tissue adhesive vet-bond (3M), the skull was attached to a head holder and the ear bars were then removed to free the visual field (see panel A in figure 1). In these experiments, the animals breathed spontaneously with a flow of pure oxygen (0.5 l/min) directed to the nose. All animal received 5 ml (i.p.) of saline solution (0.9%) every six hours to prevent dehydration. In order to map the SC, the overlying cortex was removed by aspiration with an irrigation/aspiration fine plastic needle (Tuberculin syringe) connected to a thermotic drainage pump (model N 765-A). Data acquisition started after a one hour recovery period. The same chamber filled with agar was used. At the end of the experiment, the animal was killed by an overdose of inhaled Isoflurane.

Optical Imaging

For optical imaging, images were captured using a cooled slow-scan CCD camera (12 bits Dalsa camera 1M60) using the Imager 3001 Lab Interface (Optical

Imaging Inc). Cortical and sub-cortical surface were illuminated by green light to visualize the vascular pattern. After acquisition of a surface image, the camera was focused 800 μm below the pial surface, and then the visual cortex or SC was illuminated with halogen (Optical Imaging Inc) or mercury arc lamps (X-Cite 120 EXFO) with a 630nm filter. For both lamps, fluctuations of the light emitted caused a major source of signal blurring that prompted us to develop a light correction method presented thereafter.

Visual stimuli and acquisition

Frames were acquired at an 8 Hz rate during 10 min. The visual stimulus was back projected with an NEC projector (Model LT 155) onto a screen subtending 180 x 100 degree of visual angle and placed 25 cm away at an angle of 45 degrees to the eye contralateral to the imaged hemisphere. The cortical and sub-cortical responses were recorded using the imaging method developed by Kalatsky & Stryker 2003. In this method, a temporally periodic stimulus is continuously presented to the animal. The stimulus consisted of a 100% contrast light bar (3 degrees width), horizontally or vertically oriented, drifted on a dark background at a fixed frequency (0.08 to 0.33Hz).

Signal analysis and image processing

To remove the contribution of light fluctuations, raw data was corrected for each pixel (i, j) with:

$$Rc_{i,j,f} = \frac{\left(R_{i,j,f} - \left[S_{i,j} \times \frac{T_f}{\overline{R}} \right] \right)}{S_{i,j}}$$

where $R_{i,j,f}$ and $Rc_{i,j,f}$ are the raw and corrected data. T_f is the light time course, define as:

$$T_f = \frac{1}{XY} \sum_{i=x}^{x+X} \sum_{j=y}^{y+Y} R_{i,j,f}$$

where x, y, X and Y are the position and the size of the region of interest used as a noise reference. $S_{i,j}$ is the map of light average, define as:

$$S_{i,j} = \frac{1}{F} \sum_{f=1}^{F} R_{i,j,f} ,$$

and $\overline{R}$ is the light average defined as:

$$\overline{R} = \frac{1}{F} \sum_{f=1}^{F} T_f$$

where F is the number of frames. The reference region was chosen as a zone with no visual activation, low vascular noise (e.g. no blood vessel) and a high level of illumination. Then this signal was decomposed by Fourier transform to obtain phase and relative magnitude matrixes ($\varphi_{i,j,n}$, related to visuotopy and converted in degrees of visual angle and $A_{i,j,n}$, in percent of variation) as described in the paradigm of Kalatsky and Stryker (2003):

$$Rc_{i,j,f} = \sum_{n=1}^{N} A_{i,j,n} \cdot \cos\left(2\pi f_n t + \varphi_{i,j,n}\right)$$

Visuotopic responses were chosen at the frequency of stimulation n allowing the acquisition of $\varphi_{i,j}$ and $A_{i,j}$ maps. When the distribution range of $\varphi_{i,j}$ reached an

acceptable value (more than 50% of the visual field represented in the visuotopic map), a cortical magnification factor (CMF) was evaluated on each pixel by using a new method based on the reversed local gradient (see figure 4). First, the blood vessels and the high frequency 2D noise was reduced using low pass filtering:

$$\varphi^{G}_{i,j} = G_{\sigma} \otimes \varphi_{i,j}$$

where $\varphi^{G}_{i,j}$ and $\varphi_{i,j}$ are the filtered and unfiltered visuotopic maps and G_{σ} a low pass kernel with a cut-off σ = 150µm. Then the local gradient was computed using:

$$\nabla\varphi^{G}_{i,j} = \sqrt{\left(\frac{d\varphi^{G}_{i,j}}{dx}\right)^{2} + \left(\frac{d\varphi^{G}_{i,j}}{dy}\right)^{2}}$$

where x and y are the image axes in µm and $\nabla\varphi^{G}_{i,j}$ is the visuotopic gradient in deg/µm. Finally, the CMF (in µm/deg) was considered as the reverse of the local gradient and computed as:

$$CMF_{i,j} = \frac{1}{\nabla\varphi^{G}_{i,j}}$$

The borders of the region of interest (ROI) were used to calculate an "ovality Index" corresponding to the ratio between the height and width of the ROI.

RESULTS

Signal components.

In most experiments, fluctuation of the emitted light provoked a strong attenuation of the physiological responses precluding any quantification. A representative example is shown in Figure 1 from cortical recordings. Panel B illustrates the

Fig 1 near here

temporal profile of the optic signal before and after correcting for light fluctuations (the sudden changes of light levels are indicated by the arrows in graph B1). The Fourier transforms of the signals are shown in panel C. The spectrum for the uncorrected signal was rather complex and the light instability components (mainly distributed in the low frequency range) masked all physiological signals. After correcting the signal, all physiological periodic components could be seen and measured (panel C2). Two vasomotion components were observed (V, inset). The first, made of several peaks, appeared at a mean frequency (±SD) of 0.089 ± 0.018 Hz but was not consistently observed across experiments. The second single-peaked was located at a mean of 0.042 ± 0.006 Hz (clearly visible in the temporal space in panel B2). Vasomotor components were also observed in the SC. Respiration components (R in panel C2) were also clearly visible at 1.6 Hz (96 resp/min) and represented an important part of the signal. The heartbeat component was also evident (H). Finally, the stimulus frequency (S), i.e. the visual response, was located at 0.33 Hz. The amplitude of this signal varied considerably across animals (mean ± SD of 4.9 ± 2.7 10^{-5} %) and was optimal for stimulus frequencies ranging between 0.17 and 0.33 Hz. Therefore, the physiological noise came from respiration and vasomotor components and to a lesser extent, heartbeat components. The amplitude of these components was several hundred times greater than that of the visual signal. The consequences of the above described corrections are illustrated in panel D which show the region of interest (ROI) comprising the primary visual cortex (V1). In panel 2, the fluctuations of light dominated the signal, yielding a visuotopic map

(elevation) of very low quality. After correction, there was a noteworthy enhancement of the visual map (panels 3 and 4).

Visuotopic mapping

Fig 2 near here

The periodic stimulation paradigm was used to acquire azimuth and elevation visuotopic maps in response to vertical and horizontal bars, respectively. Panels A and B of figure 2 shows the change of the visual field representation along the azimuth and elevation on the surface of V1 in a pigmented rat. The naso-temporal progression of the visual field was represented along the latero-medial axis (panel A) while the superior-inferior axis was represented in the caudo-rostral axis of the cortex (panel B). A comparable mapping profile was obtained in albino rats (panels C and D). The activated region was always elliptic (mean ovality index ±SD = 0.72 ± 0.08) and had a mean area (±SD) of 5.02 ± 2.48 mm^2.

In some cases, a response was also present at the second harmonic level (panel E). The corresponding visuotopic map shown in panel F was characterized by better-defined boundaries. However, the use of the second harmonic duplicates the visuotopic map, yielding incertitude in the visual position represented (± π). Nevertheless, second harmonic analysis can be exploited to improve the limit of the targeted structure and the CMF measures (see below). In one experiment, the activation of V1 was accompanied by the activation, albeit weaker, of a smaller zone lateral to the main functional map, and assumed to be V2 (figure 3). Along the azimuth, this second map showed a transition from lateral to medial, therefore being the mirror image of the visuotopic arrangement observed in V1 (panel A). The

elevation map however showed a similar progression along the caudal to rostral axis in both activated regions (panel B).

Superior colliculus. The SC of two pigmented rats was successfully imaged. Given that the overlying cortex had to be removed by aspiration (trauma), optical responses were generally weaker and blood was often present at the collicular surface. Nevertheless, we were able to acquire visuotopic maps as shown in panels C and D of figure 3. As seen in V1, the functional maps along the azimuth (panel C) traveled from the lateral to medial axis while the elevation maps progressed from the caudal to rostral part of the SC (panel D).

Fig 3 near here

Cortical magnification factor. For the cases showing a clear cortical visuotopic arrangement (as in panels A1 and B1 in figure 4), a continuous quantification of the CMF was performed (Figure 4). A low pass filtering was first applied (panels 2), followed by a measure of the local gradients i.e. a measure of the changes of the visual representation on a delimited cortical area (panels 3). A high gradient would indicate a large variation of these changes. Then, the inverse of the gradient was computed and corresponded to the CMF i.e. the changes of cortical surface as a function of the stimulus position in the visual field (panels 4). In the first example (panel A), the progression of the visuotopy along the azimuth was greater in the center of ROI leading to a higher gradient and a lower CMF. The same profile was observed for the elevation map (panel B).

Fig 4 near here

To validate the CMF method, the azimuth and elevation maps were displayed in blocks of 10 x 8 degrees (panel C). We assume that the resulting representation is comparable to the one that would have been obtained with the standard episodic method. As observed for azimuth and elevation continuous CMF maps, activation zones were smaller in the center of the ROI than in the periphery, supporting the findings presented in panels A and B.

The above data confirmed that the continuous CMF can be used to reveal and quantify the changes in cortical topography. In most experiments, the CMF maps were homogeneous throughout the cortex as shown in panel D. Overall, the mean CMF value was 44 ± 12 μm/deg. Finally, when the surrounding 2D noise (see figure 2, panel D) was prominent, the CMF values were over- or under estimated according to the zone considered. A solution was to quantify the CMF from the maps obtained at the second harmonic. An example is shown in panel E. The map at the first harmonic was not homogeneous leading to uncertainty about its limits (panel E1). This was not the case for the second harmonic as the corresponding map appears uniform (panel E2).

DISCUSSION

This study showed that the continuous imaging paradigm developed by Kalatsky & Stryker (2003) can be successfully used to map the primary visual cortex and SC of pigmented and albino rats (data not shown). Thanks to this paradigm, visual maps can be obtained in a short time period (10 minutes); this last aspect being critical

when investigating the immediate effect of experimental manipulations. It also showed that a major source of noise comes from light fluctuations. This problem was overcome by developing a correction method which considerably improved the signal. Finally, continuous visuotopic maps were characterized and exploited to develop a measure of local cortical magnification, the CMF. The development of a pixel-wise measure of CMF presents several interests. This quantification can be particularly useful to determine very detailed visuotopic patterns that would not be noticeable otherwise. Also, a major advantage of the CMF measure is to be able to characterize circumscribed (i.e. selected region of a visual structure) and relative (i.e. uncorrected by the hemodynamic delay) visuotopic maps.

Technical considerations. In the first steps of this project, imaging the visual cortex of the rat proved to be difficult compared to other animal species used in the laboratory such as mice, cats and tree shrews. Anesthesia was found to be an important factor. Initially, animals were anesthetized with a gas mixture of O_2 and Isoflurane. In contrast to halothane, another volatile anesthetic, Isoflurane has little impact on cardio-vascular function and intra-cranial pressure (Drummond et al., 1983; Todd and Drummond, 1984; Frost, 1984), making this drug a good candidate for anesthesia for rat optical imaging experiments. This was not the case as no reliable maps of the primary visual cortex could be obtained under Isoflurane anesthesia. This anesthetic is known to exert a strong depressive effect on cortical responses in cats (Villeneuve and Casanova 2003) and it may have the same detrimental effect in rats. Isoflurane was abandoned and anesthesia was induced by

Urethane even if this drug has been shown to depress cortical activity in rats (Girman et al., 1999). On the other hand, urethane is known to exert minimal effects on cardiovascular and respiratory systems (Hara and Harris 2002; Koblin 2002). Switching to urethane-based anesthesia was effective as it became easier to acquire stable and reproducible functional maps of the primary visual cortex.

Light fluctuations represented the major source of noise in our experiments, as it might be for other laboratories, despite of our efforts to address this problem in a practical way (e.g. two technologies tested: halogen and arc lamps, stabilization of the power supply, additional cooling of the lamp). In presence of this noise, no quality or robust maps could be obtained and quantification was almost impossible. While Fourier decomposition is a suitable method to analyse periodic signals, non-periodic events such as light instabilities are quite complex to resolve in the frequency domain. By selecting a reference profile of light fluctuations, we were able to correct the raw signal and strongly enhance the visual responses. The main problem of this signal correction lies in the choice of the region of interest to be considered as noise reference. In an ideal situation, this region should not have any physiological signals, and that was virtually impossible in our case given that the skull was not present in the field of view. Nevertheless, the use of a non visual part of the cortex allowed us to adequately remove the fluctuations with little interference on physiological signals In contrast to light instability profiles, each of the physiological components are not appearing at the same moment in the different parts of the cortex.

The second main source of noise to deal with came from the animal respiration. This noise may have originated from breathing movements passed on to the skull since the animal's head was only secured by a head holder. In support of this assumption, respiratory signals were barely noticeable in our cat preparations (with the head more firmly held by mouth and ear bars; data not shown). Nevertheless, this noise was not critical as it did not overlap with the signal frequency. The third source of noise came from the constriction/dilation of arterial blood vessels. Vasomotor oscillations are happening spontaneously at a frequency lower than that of respiration (Japundzic et al. 1990; Mayhew et al. 1996). Despite initial worries, we found that vasomotion was not a major source of noise. These noise components were not present in all experiments and their frequency (generally peaking at both ~0.04 and ~0.09 Hz) was lower than that of the visual signal. Despite the invasive surgery necessary to uncover the SC, vasomotion was also recorded in this structure, suggesting that the surgical procedure did not alter its local blood flow.

The stimulus frequencies used in the present study were thus chosen so that they will not overlap with respiratory and vasomotor components. The best responses were recorded between 0.17 - 0.33 Hz. In a parallel study, we determined that such frequencies are indeed ideal to maximally exploit the signal revealed via the hemodynamic response (Vanni et al. 2007).

Visuotopic maps in the visual cortex and superior colliculus. To our knowledge, only Gias et al. (2005) characterized the visuotopic organization of the visual cortex

with OBI in normal rats. Our findings are comparable to theirs as they showed the existence of an orderly representation of the contralateral visual field, characteristic of that obtained by electrophysiological mapping (Montero et al. 1973). A difference between Gias et al. (2005) study and ours is the method used to acquire the maps. These authors used the conventional episodic method which necessitates the averaging of the signals, increasing substantially the recording period (our experience is that a visuotopic testing period would last hours instead of minutes; data not shown) and reducing the resolution of the visuotopic maps. In theory, maps obtain with the periodic stimulation paradigm could be made from an infinite number of points of the visual field (Kalatsky and Stryker 2003).

In all functional maps recorded in the present work, the contour of the primary visual cortex was elliptical, in accordance with previous studies based on electrophysiological recordings (Montero et al. 1973; Espinoza and Thomas 1983; Rumberger et al. 2001). However, the surface of the activated regions was smaller (around 2 mm^2) than that reported by others (Espinoza and Thomas 1983; Rumberger et al. 2001; Gias et al. 2005). This is likely due to the fact that our setup did not allow for the stimulation of the more temporally located part of the visual field. Also, since we used a flat screen, the bar in the extreme nasal and temporal part of the screen may have appeared thinner and slower to the animal and thus may have been less optimal to stimulate the corresponding regions of the cortex. It is worth mentioning that, in this project, we did not intend to obtain a mapping of the entire visual field, but rather determine the usefulness of the continuous stimulation paradigm in rat and develop tools for noise reduction and magnification

quantification. Another possibility for the underestimation of the cortical surface comes from the fact that, in some cases, the stimulus frequency was close to the vasomotor components. In these cases, a 2D vascular noise pattern was superimposed on the 2D visuotopic pattern yielding an incomplete estimation of the limits of the activated area, thus inserting additional variability between the estimation of the cortical surface across animals. This problem was solved by using visuotopic maps from second harmonic signals. It is indeed known that noise components (such as vasomotion) are virtually absent at higher frequencies (Mayhew et al. 1996).

In all but one experiments, no significant activation beyond area V1 was seen. For one animal, a second visuotopic representation, smaller in size and amplitude and lateral and slightly posterior to the primary visual cortex was detected, comparable to that found by Gias et al (2005). Based on its position and dimension, this region is likely to correspond to area V2 (Adams and Forrester 1968; Espinoza and Thomas 1983; Montero 1993; Rumberger et al. 2001). The lack of consistent and reproducible responses from the assumed V2 area may come from the fact that the visual stimulation parameters were chosen to optimally activate V1 (and not necessarily V2).

Finally, the present study reports for the first time that the visuotopic organization of the rat superior colliculus can be investigated with optical imaging. Comparable data were obtained in another rodent, the mouse (Cang et al. 2005b;

Mrsic-Flogel et al. 2005; Cang et al. 2008). Our results show a similar progression of the representation of the visual field as that reported in electrophysiological mapping studies (Rhoades et al. 2000); i.e. in which the superior visual field is represented caudally and the nasal visual field is represented laterally. The amplitude of the optical signal was weaker than that of the cortex, perhaps because of the removal of cortico-tectal fibers (believed to exert a facilitation (Goodale 1973)) and the trauma associated with the aspiration of the cortex. The possibility to map the visual space in the SC is of great interest when considering animal models of pathologies in which ganglion cells are affected (i.e. glaucoma). Indeed, it is known that all ganglion cells directly projects in a orderly manner in the SC, allowing then an assessment of the visual processing only one synapse away from retinal ganglion cells.

FIGURES

Figure 1. Optical signal in the rat cortex **(A)** schematic representation of the recording setup. **(B)** Temporal profile of the signal recorded in one location of the cortex of an albino rat before (1) and after (2) light fluctuation correction. The visual response was evoked by a moving horizontal bar. The arrows indicate the sudden changes of light level which were removed by the correction. **(C)** Power spectrum on uncorrected and corrected signals. After light fluctuations correction, stimulation (S), respiration (R), heartbeat (H) and vasomotion (V) components can be clearly seen. Because of the sampling frequency used (8Hz), the peak for the heart was always filtered and consequently do not appear at the right frequency on the graph (the peak appearing at 3.6 is in fact located at 4.4 Hz (264 bpm), the Nyquist frequency being at 4 Hz). **(D)** (1) Vascular pattern in the region of interest. (2) Visuotopic map normalized by the amplitude of the signal (f = 0.333 Hz) from uncorrected experiment. (3) and (4) shows map before and after being normalized by the amplitude of the signal, after correction. Scale bar = 1 mm.

Figure 2. Visuotopic maps of area V1 in a pigmented rat. **(A, B)** Visuotopy map normalized by the amplitude of the signal at the stimulus frequency for azimuth and elevation. **(C, D)** Comparable maps were obtained in an albino rat. **(E)** Power spectrum displaying vasomotor components (V) and first (1) and second (2) harmonics linked to the stimulation. **(F)** Visuotopic map normalized by the

amplitude of the signal at twice the stimulus frequency (second harmonic) from the same recording presented in panel D.

Figure 3. Visuotopic maps in area V2 and in the superior colliculus. **(A, B)** Visuotopic map after and before being normalized by the amplitude of the signal at the stimulus frequency in the visual cortex of an albino rat. The arrow indicates a weak activation in a region assumed to be V2. Results are schematized in the insets. **(C, D)** Visuotopic map normalized by the amplitude of the signal at the stimulus frequency recorded from the superior colliculus of a pigmented rat. Scale bar = 1 mm.

Figure 4. Cortical Magnification Factor. **(A)** (1) Visuotopy at the stimulus frequency along the azimuth.. Method was as follows: (2) low pass filtering, (3) gradient of the filtered visuotopy and (4) inverse of this gradient corresponding to the CMF. The histogram in the inset shows that more than 80% of the visuotopic positions were represented. (B) The same procedure was applied for elevation visuotopy. **(C)** Combined azimuth and elevation visuotopy displayed in blocks of 10 x 8 degrees. **(D)** Other examples of CMF maps from first harmonic signals displayed in a logarithmic scale. **(E)** CMF maps from the first (1) and second (2) harmonics. These maps comes from the visuotopic maps in panels D and F of figure 2.

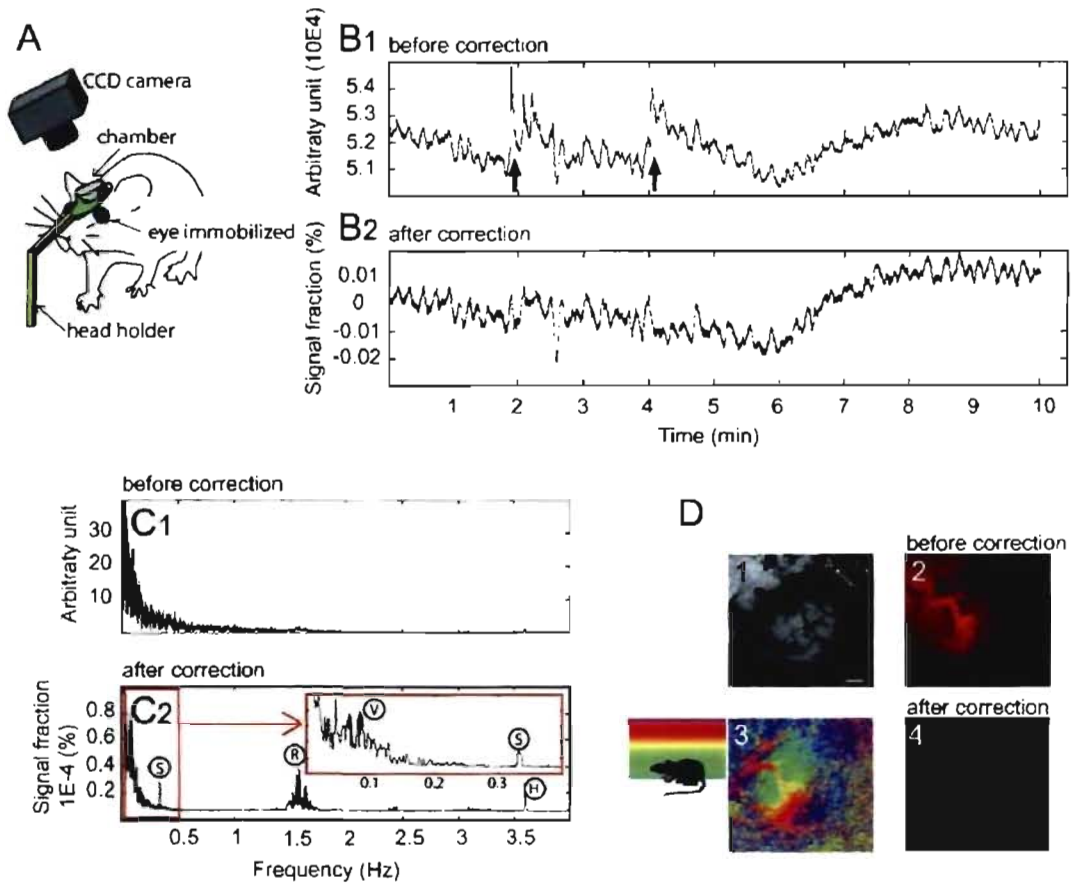

Figure 1. Nassim et al.

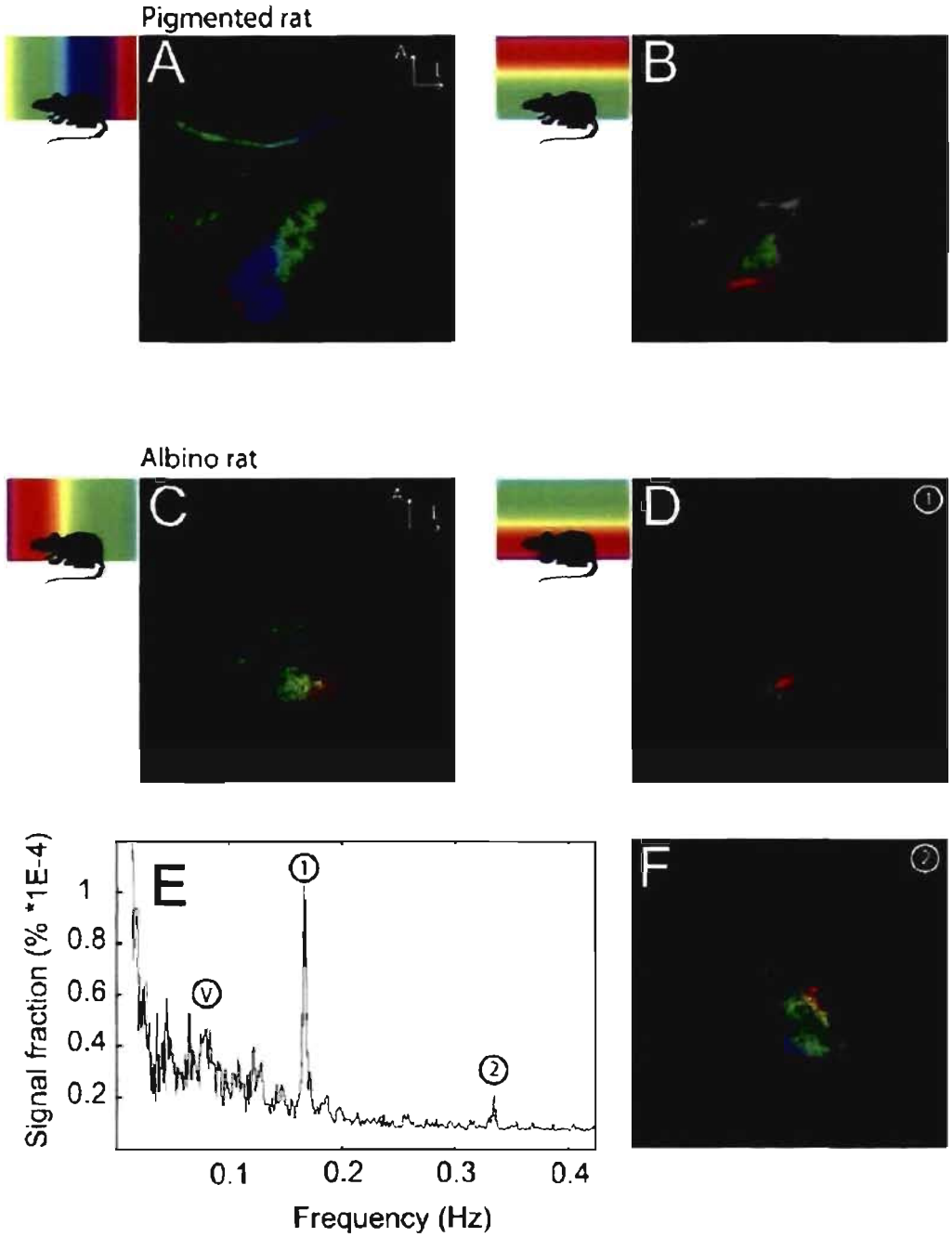

Figure 2. Nassim et al.

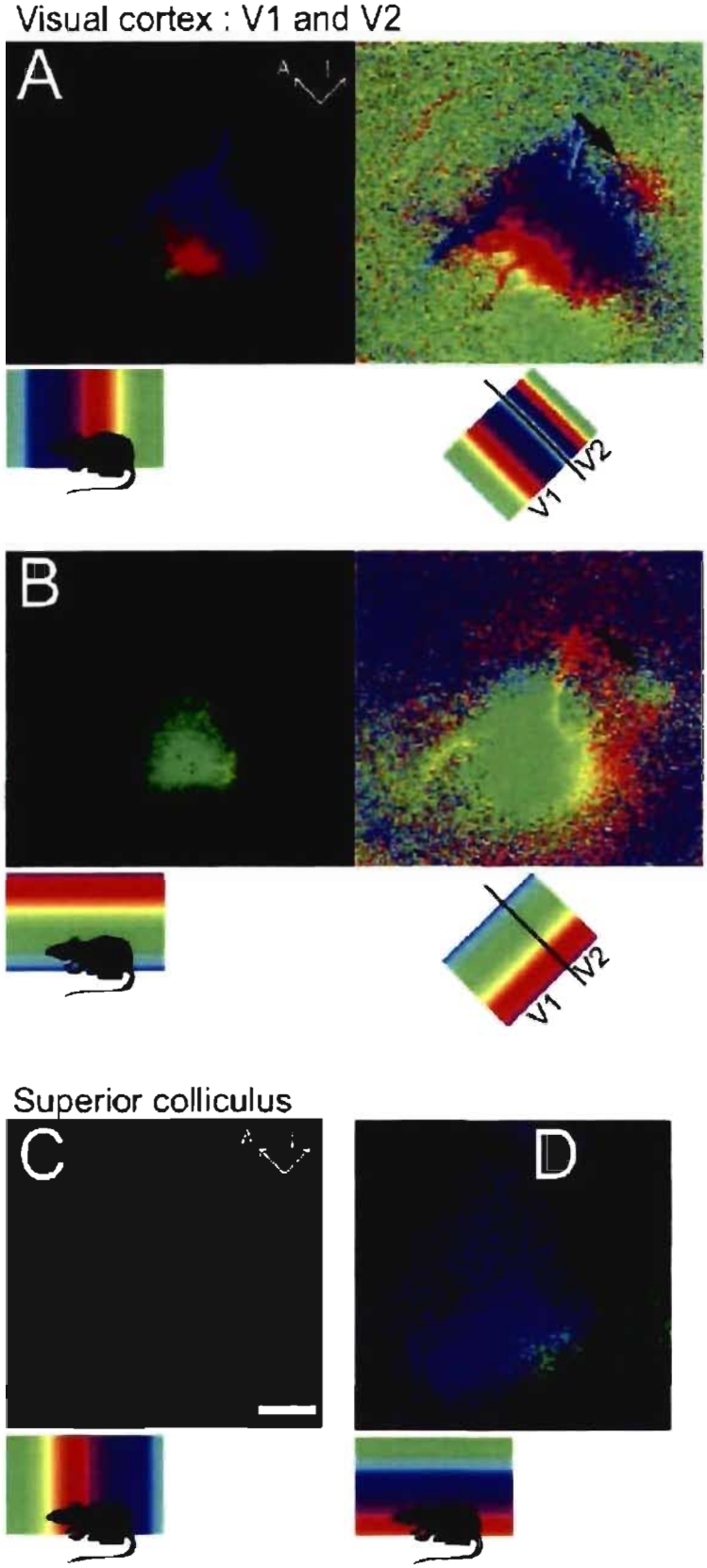

Figure 3. Nassim et al.

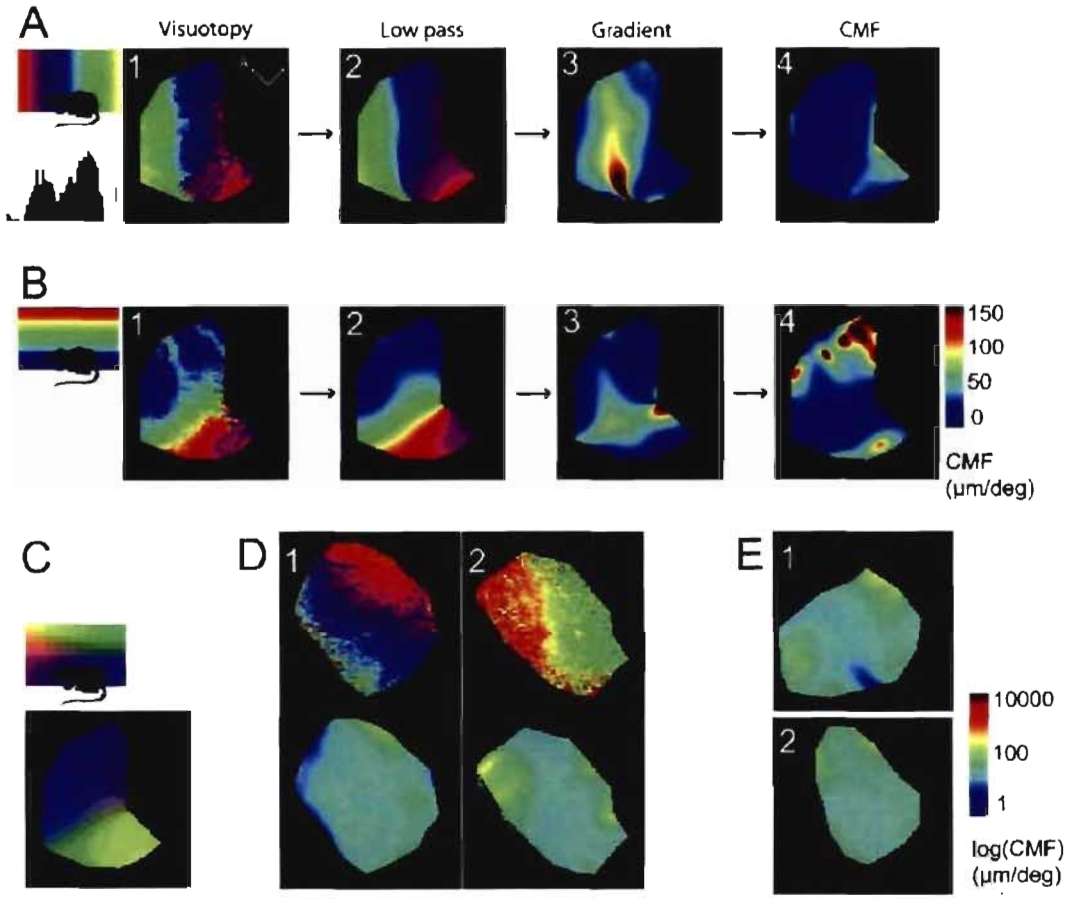

Figure 4. Nassim et al.

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DISCUSSION

1. General observations

Using continuous stimulation developed by Kalatsky and Stryker 2003, we were able to trace the retinotopic organization of the primary visual cortex and superior colliculus in both albinos and pigmented rats. Obtaining retinotopic maps allowed us to quantify different physiological parameters such as the cortical magnification factor (CMF). We found that retinotopic maps of the primary visual cortex and superior colliculus were in accordance with previous electrophysiological studies. No differences on CMF between albino and pigmented rats were detected and the distribution of the cortical magnification factor was uniform across the primary visual cortex and superior colliculus in both albinos and pigmented rats.

2. Methodological consideration

2.1 Animal surgery considerations

Before this study, most of the optical imaging experiments in our laboratory were done on cats. Cats were anaesthetized by O2/N2O /Halothane gas mixture, were ventilated, and oxygen blood saturation was monitored using oxygen saturation meter. This chirurgical paradigm showed reproducible results. When I began this study, I initially tried to anesthetise the rat with gas, and the rat's anaesthesia was maintained by inhalation of an O2/isoflurane gas mixture but not paralyzed. After

many trials, more than thirty rats, no retinotopic organization were detected of the primary visual cortex. We concluded that:

1- Inhalation by isoflurane is not an effective anaesthesia in rats and may exert a strong depressive effect on the cortical response as it was shown in cats. (Villeneuve and Casanova, 2003)

2- The stimulus paradigm and the analysis approach used in this study were not appropriate.

To resolve the first problem, we reviewed many studies done in rodents (i.e., mice and rats) and noticed that injection by ketamine or urethane was mostly used in these small animals. However, Ketamine has only a short effect, hour long; and it would not be an ideal anaesthesia for this project because of the long recording in optical imaging. Moreover continuous injection of ketamine would also be inconvenient because the animal would be on different levels of anaesthesia during the recording and this could potentially affect the vascular and neuronal response.

I did not choose to use urethane at the beginning because my aim was to compare many results with previous electro-physiology studies that used isoflurane as anaesthesia. To be able to do that we must keep the same condition, and also at beginning we did not know if urethane was the problem.

We chose to use urethane for two reasons:

1- Urethane exerts minimal effects on cardiovascular and respiratory system (Koji and Hara 2002, Koblin et al., 2002).

2- Urethane is suitable for long recording because of its prolonged effect on animals (eight hours).

In OBI experiment, Cat and monkey usually are paralyse this prevent eyes movement. However, rats were not paralyzed and the representation of the visual field on cortical maps could be affected because of eye movement. Eye movement will change the representation of the retina on the visual field. Collecting intrinsic images will not reflect the exact representation of the visual field on the primary visual cortex and on the superior colliculus. To overcome this problem we fixed the eyes with tissue adhesive vet-bond (3M). Paralysing the animal would have been ideal, but using tissue adhesive vet-bond was a viable alternative. The advantage of tissue adhesive is that no surgery procedure is needed, and it is very fast. However, the disadvantage is that if it's not done properly we could potentially covert some parts of the eye with the glue, producing only a partial retinotopic map of the primary visual cortex or SC.

2.2 Illumination and source of intrinsic signal

During my project I tried many wavelengths from 580 nm to 700 nm, however I noticed that the best quality of the functional map occurred when I used a wavelength between 600-630nm. This wavelength detected a mix of CBV and Hb/Hbr changes. Others studies have used different wavelengths like 700 nm. This may be due to the fact that the best source of intrinsic signal in OBI depend on cortical area being imaged (area 17, 18,21), animal (rat, cat, mice...) and also the

difference across species concerning noise (Ex: Mayer waver which is bigger in rat).

2.3 Conventional method in optical imaging

Initially we tried to map the primary visual cortex using conventional methods by employing different stimulus designs and image analysis approaches (Fist Frame Analysis, Z score). We started by stimulating the animal eyes using two stimulus paradigms:

1- Sine wave grating stimuli in two different locations of the visual field

2- Sine wave grating stimulus in six different locations of the visual field.

The duration of each stimulus was three seconds, followed by a five second relaxation time. The recording duration was approximately one to three hours and the average frames of each trial were stored separately on the computer's storage device. We then analyzed them using the first frame analysis in which the pre-stimulus frames (blank) were subtracted from the stimulus frames. Our goal was initially to see if we could detect any neuronal activity. After many trials and more than fifteen rats, we could not map the primary visual cortex retinotopy. Furthermore, we could not detect any neuronal activity.

We tried another image analysis approach to improve the ratio signal/noise. For that we used the Z score approach in which every pixel in each frame was transformed into Z score value by removing the average of the pre-stimulus frames from the

whole frames and dividing the result by the standard deviation of the pre-stimulus frames (Gias et al., 2005).

$$Z(x_i, y_i, t_j) = \frac{I(x_i, y_i, t_j) - \text{mean}(I(x_i, y_i, T_1))}{\text{std}(I(x_i, y_i, T_1))}$$

xi and yi represent the spatial coordinates of the ith pixel, tj is the jth time sample, T1 represents the pre-stimulus interval and I is the raw pixel's time series (Gias et al., 2005).

Unfortunately, even with these changes no neuronal activity was detected. The major problem was the noise, and we concluded that we had to find more developed image analysis approach. Another approach called Principal Component Analysis (PCA) transforms the data frames to a new coordinate system in which the biggest variance by any projection of the data lies on the first coordinate (called first principal component). In optical imaging data, the PCA positions all frames on a new orthogonal set of images according to their variance (i.e. signal strength) then the noise will be selected, (Schuett, et al., 2001). This method will be developed in our laboratory for future projects.

In sum, more than forty rats were tested to map retinotopy within the visual cortex using the conventional method. Unfortunately we were unable to map retinotopy maps using this method because in optical imaging the signal represents less than 0.1% of the reflected light, and since only the average responses are stored, it is difficult to separate the "noise" fluctuations in reflectance from the signal of neural activity.

We concluded that conventional methods in optical imaging based on representing periodic stimulus were not suitable in this study for many reasons:

1- Required more than three hours to map only one structure (Gias et al 2003, 2007) and is incompatible with the recording of multiple brain areas in the same anesthetised rat.
2- Rat Mayer waves have big amplitude and cause artefact. It would be hard to eliminate it by simply averaging the data. Moreover, the noise is not periodic so averaging data is not suitable is this case.
3- Choosing a limited stimulus position in the visual field: (a) reduce the spatial definition (B) and cover a non complete range of stimulation.

To remedy these problems we adapted a new stimulation paradigm that was developed by Kalatsky and Stryker 2003, called continuous stimulation and spectral decomposition.

4. Continuous Stimulation and spectral decomposition

4.1 Continuous Stimulation

One of the goals of this study was to build a new stimulus in our laboratory based on continuous stimulation and to develop new quantification methods of CMF based on gradient technique

This technique offers the advantage of reducing the acquisition time to ten minutes, allowing the possibility for multiple tests to be performed on the same

animal (i.e. using different spatial frequencies, different stimulus design: moving spot, rotating/ horizontal/vertical stimulus, or to map different structures etc.). In the mouse, for example, many efforts have been made to study the effect of short eye deprivation on animal acuity and ocular dominance (Heimel 2007) which requires testing many stimuli with different spatial frequency. This technique is advantageous because all frames are saved. Saving full-time series allows us to extract the neuronal signal from all signal components like heart beat (2–5Hz), respiration rate (0.3–1 Hz), vasomotor signal (0.05–0.1 Hz) (Mayhew et al., 1996), and slow variation of the baseline due to the state of a subject. However, saving all frames requires us to increase the storage space of our data acquisition computers.

This new stimulus paradigm was designed to excite all neurons at successive phases of the stimulus. However, the phase maps obtained will be relative maps because they will be shifted by the haemodynamic delay. The hemodynamic delay is evident from the fact that the stimulus leaves the receptive field before the hemodynamic changes occur. To avoid this problem one must correct the map by subtracting the haemodynamic delay from the phase map (For further explanation see the section entitled "Discussion: Primary Visual Cortex.")

4.2 Fourier analysis

In optical imaging data, the frequency of stimulation is extracted from a complete time series of image intensities acquired at each pixel. The frequency of stimulation (0.166Hz-0.33Hz) in this study was chosen so as not to coincide with

system internal oscillation (respiration, heart beat, and Mayer waves). The advantage of Fourier decomposition is:

1- It is possible to remove the response components corresponding to the various stimulus frequencies.

2- We may also use two different stimuli with different periods (Kalatsky et al., 2007). For example, we can map the azimuth and elevation retinotopic map by using a vertical and horizontal bar at the same time but with different frequencies. Then, we can extract the signal of each map.

Using the new paradigm and other chirurgical modification cited above, we were able to map the retinotopy of the visual cortex. However, the maps were not stable, and not easily replicated.

5. Visuotopic maps in the visual cortex and superior colliculus in rats

The primary visual cortex presented a distinct and precisely arranged retinotopic organization. This organization was demonstrated with anatomical and electrophysiological studies (Montero et al., 1973). Recently, Gias et al. 2005, 2007 characterized the retinotopy of the rat visual cortex in non-dystrophic rats using conventional methods of brain optical imaging. In this study we tried the conventional methods but were unsuccessful. Continuous stimulation was used in this study.

5.1 Signal amplitude and rat acuity

In order to study the effects of albinism on rat visual function, one of our aims in this study was to measure and compare the rat acuity in both albino and pigmented rats. Previous behavioural studies found that strain selection associated with albinism appears to have consistently impaired visual acuity (Prusky et al. 2002).

To address this question using optical imaging technique, we tried to measure the magnitude of the intrinsic signal (neuronal signal amplitude) while stimulating the animal's eye with various stimuli with different spatial frequencies (0.3-2 cycle per degree). The results found were discouraging and it was difficult for us to quantify the signal magnitude. This issue may be the result of:

1- The stimulation frequency: being close to the Mayer wave frequency when we tested different stimulation frequencies may obscure the amplitude of the neuronal signal.

2- Illumination stability: this depend both on the nature of the light source, its power supply and the connections between the power supply and the bulb.

3- To remove the contribution of the instability of illumination, we corrected our raw data with :

$$Rc_{i,j,f} = R_{i,j,f} - \left[\left(\frac{1}{F} \sum_{f=1}^{F} R_{i,j,f} \right) \bullet \frac{\left(\frac{1}{XY} \sum_{i=x}^{x+X} \sum_{j=y}^{y+Y} R_{i,j,f} \right)}{mean(R)} \right]$$

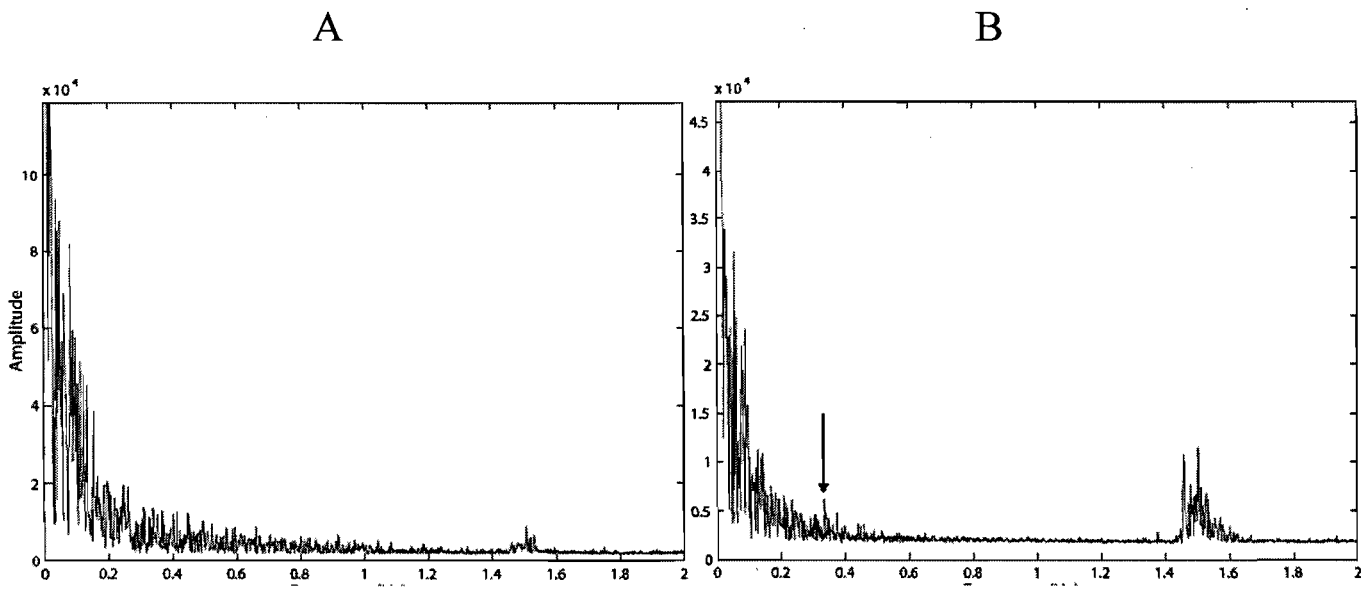

Figure 11. Removing the contribution of the instability of illumination in intrinsic signal at single pixel from a series of frames of the rat primary visual cortex. (A): original signal without removing the contribution of the instability of illumination. (B): signal after removing the instability of illumination. Arrow indicates the neuronal signal.

Where R and Rc are the raw and corrected data, x, y, X and Y are the position and the size of the region of interest used as a noise reference and F is the number of frames. The reference region chosen does not have visual activation, high vascular noise (e.g. blood vessel) and low illumination levels.

Removing the contribution of the instability of illumination in our signal has indeed ameliorated the amplitude signal in some of our data (figure 11). We believe that further analysis of our data is necessary to validate this approach.

5.2 Primary visual cortex retinotopy

The size of the primary visual cortex found in this study was around 7-8 mm in agreement with the stererotaxic coordinate of paxinos. The quantification of the primary visual cortex size was done manually using adobe illustrator software. This quantification method was not the best solution because it was established by approximations. However, the borders of the primary visual cortex were detected solely by the progression of the color code in the retinotopic maps. Alternative methods would be more appropriate, like using more advanced analyzing techniques that can detect the activated region.

The retinotopic organization of the primary visual cortex in both albino and pigmented rats were in accordance with previous studies in which the superior visual

field (lower retina) is represented caudally and the nasal visual field (temporal retina) is represented laterally (Adams and Forrester, 1968; Espinoza and Thomas, 1983; Montero et al., 1973, Gias et al., 2003)

The phase maps shown in this study are relative maps because they are shifted by the haemodynamic delay. Nonetheless, these maps of the relative retinotopy are enough to quantify cortical magnification factors or to study the relation of retinotopy with other cortical functional structures.

Having obtained a retinotopic map, we were able to quantify the cortical magnification. The result found were in accordance with previous electrophysiology studies (Espinoza and Thomas 1983). More ever, we found that there is no significant difference in CMF across the primary visual cortex in both species. Contrary, electrophysiological studies reported that there were variation in the CMF across visual areas in the rat but were much smaller than those found in higher mammals (Adams and Horton, 2003; Van Essen et al., 1984).

In both albino and pigmented rats, there was no difference between horizontal CMF and vertical CMF within the primary visual cortex. In the rat, many studies have shown that there is a variation on the distribution of rat ganglion cells across the rat retina, from the highest to the lowest density, from only 3000 to 600 cells per mm2 (McCall et al., 1987). Using intrinsic optical imaging techniques we were unable to detect these differences across the visual cortex, and the CMF were similar in both species. Most of the studies performed in higher mammals, which have a fovea, have suggested that the ganglion cell number across the retina matches the distribution of the corresponding magnification factor in cortical and subcortical

structures (cortex: Wässle et al.,1989; superior colliculus: Mark et al., 1993, Rosa and Schmid, 1995).

In rats, studies have reported many extrastriate areas, Oc2L (18a) and Oc2M (18b) (Espinoza and Thomas, 1983; Montero et al., 1993). Others argue that only one or two extrastriate visual areas exist (Cusick and Lund, 1981; Malach et al., 1989; Rumberger et al., 2001). In our study we could detect in one animal a putative extrastriate activation posterior and lateral to area V1 which may correspond to V2 because of the mirror representation of the visual field (Fig 12). Being unable to detect V2 in every case may be due to the fact that V1 and V2 may be stimulated with different spatial frequency stimulus. For future projects it will be important to confirm V2 by immunohistochemistry because we believe that an optical imaging approach would be useful to determine the number and organization of extrastriate areas in rats like it was done in mice with the same paradigm (Kalatsky and Stryker 2003, Schuett et al., 2002). We also believe that using different spatial frequencies can activate others extrastriate areas.

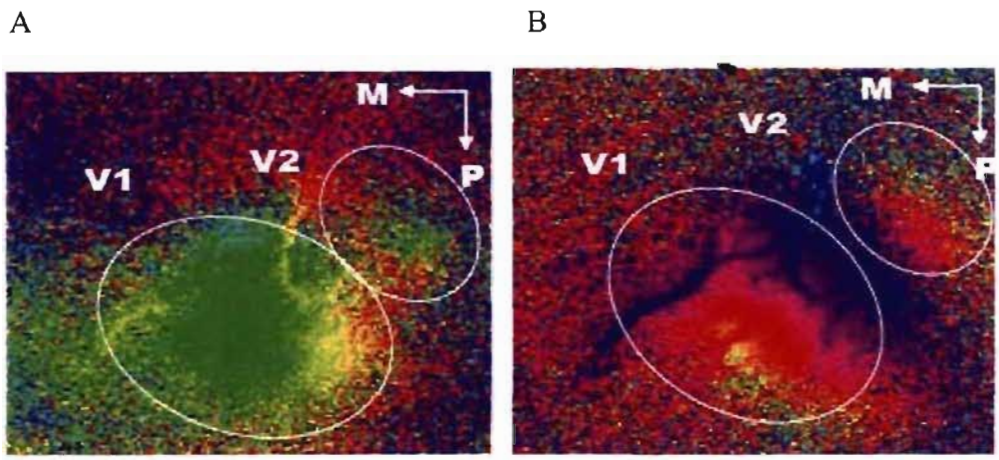

Figure 12: Retinotopy phase map of V1 and V2 in pigmented rat using continuous stimulation. A: Elevation retinotopy. B: Azimuth elevation. P: posterior. M: medial. Color code indicates specific position of the stimulus in the visual field

5.3 Superior colliculus

As far as I know this study is the first one to investigate the retinotopic organization of the superior colliculus using OBI. The superior colliculus was exposed by aspiration of the visual cortex. The consequences of removing the overlying cortex are loss of blood and the inability of the SC to resume its normal condition only one hour after the surgery. However, many studies have proven that the ablation of the visual cortex does not affect the organization of the superior colliculus because the cortico-tectal projections have merely a facilitation role (Goodale et al., 1973). A unilateral ablation of the primary visual cortex by aspiration or cooling produced a depression of the light-evoked potential recorded in the ipsilateral side (Goodale, et al., 1973). In our case this may affect the intrinsic signal amplitude but does not affect the retinotopic organization within the superior colliculus.

The retinotopic organization found on the superior colliculus was in accordance with previous electrophysiological studies in the rat. The nasal visual field is represented on rostral part of the SC, whereas the temporal visual field is represented on the caudal part of the SC, upper visual field are located medially and lower visual field are located laterally (Cynader and Berman 1972; Schwassmann 1996).

6. Future applications

Mapping superior colliculus and visual cortex in normal rats and quantifying the CMF with high precision will provide an excellent opportunity to functionally assess retinotopic maps in injured rats.

In the past few decades, rats have been widely used as an animal model for human retinal diseases such as age-related macular degeneration, glaucoma, and retinopathy of prematurity. Many anatomical and electrophysiological studies have investigated the impact of retinal degeneration on cortical and sub cortical structures. For example, Sauvé et al., 2003 have investigated the impact of glaucoma on superior colliculus by studying the size of the receptive field using multi-electrode recording. They found that in glaucoma models the size of the receptive field was larger than normal. We believe that optical imaging can provide more information concerning the size of the receptive field. Using continuous stimulation, higher harmonics in intrinsic signal can provide more information about the neuronal proprieties.This new approach will be useful in studying the effect of retinal degeneration on representations of the visual field on cortical and sub-cortical area. Also the OBI can be a great tool to study the function of different proteins in the nervous system and to understand the mechanisms that controls the development of retinotopic maps in the brain (e.g. Ephrins, Drescher et al., 1995).

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