Orientation selectivity of neurons and their spatial layout in cat and agouti primary visual cortex

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Abstract

So far, there is no evidence of columnar orientation preference maps in rodent primary visual cortex, such as commonly observed in carnivores and primates. Nevertheless, orientation selective neurons have been found in all rodent species investigated, though interspersed. This opens up the question whether the connectivity underlying the emergence of selective cortical response properties in animals with interspersed as compared to columnar maps follows a different blueprint. Rodent data are so far mainly available for species with nocturnal or crepuscular habits and small brain size, two factors that could also contribute to develop a different functional architecture.

Therefore, we set out to compare the functional architecture of the primary visual cortex of carnivores with that of a big rodent with diurnal habits, and a V1 size comparable to cats and small primates. We performed multi-site electrophysiological recordings using spatial arrays from both anesthetized cats' (*Felis catus*) and agoutis' (*Dasyprocta aguti*) visual cortex. Visual stimuli consisted of contrast reversing checkerboards and oriented gratings of several spatial and temporal frequencies.

Agoutis presented smaller orientation selectivity indices (median OSI = 0.10) than cats (median OSI = 0.19), and lower proportions of orientation (~45% for agouti V1 and ~75% for cat A18) and direction (~35% for agouti V1 and ~65% for cat A18) selective neurons. In order to describe the functional architecture based on the electrophysiological data, we quantified the orientation preference similarity between neurons according to the cortical distance between them. This analysis revealed a characteristic slow decrease in neuronal orientation preference similarity for cats. No such “classical” modularity was found for agoutis, but a clustering of neurons with similar orientation preference was observed for short ranges (< 250 μm).

Overall, our results are consistent with recent literature reporting ‘mini-columns’ of orientation preference in mice, and therefore further prove that the rodents’ interspersed maps are not random, as previously assumed. We refute, however, recent theoretical literature suggesting that agoutis might have “classical” columnar orientation preference maps. Future research should focus on understanding the circuits, which lead to small selective receptive fields in agoutis and great visual performance while adopting a different functional architecture.
1. Introduction

Of all the sensory systems available to organisms, vision is arguably one of the most powerful. It helps organisms interact with the surroundings in ways that other senses cannot, by letting the organisms perceive signals (light) from afar and process them very rapidly, which can save a prey’s life when detecting shadows that move overhead, as exemplified in the escape response of the crab *Neohelice granulata* (Tomsic et al, 2017). In more sophisticated implementations of vision, organisms are capable of detecting light which sources are literally light years away and use that information as an effective navigation system documented already over 2.5 millennia ago:

‘The wind lifting his spirits high, royal Odysseus spread sail—gripping the tiller, seated astern—
and now the master mariner steered his craft,
sleep never closing his eyes, forever scanning
the stars, the Pleiades and the Plowman late to set
and the Great Bear that mankind also calls the Wagon:
she wheels on her axis always fixed, watching the Hunter,
and she alone is denied a plunge in the Ocean’s baths.
Hers were the stars the lustrous goddess told him
to keep hard to port as he cut across the sea.’
The Odyssey. Homer. Translation by Robert Fagles.

But with great power comes great processing complexity and cost. In mammals, stimulus processing involves several stages.

1.1 Basic organization of the visual system

It all starts once the light hits the retina in the eye. Here, an orderly neural network formed by different cell types (i.e. photoreceptors, bipolar cells, ganglion cells, horizontal cells and amacrine cells) transforms the electrical signals generated by photoreceptors into action potentials that will be transmitted through ganglion cell
axons to the brain (Purves 2004). The intraretinal circuitry is, by itself, already a highly efficient processing center, sending to the brain encoded information about color, contrast and light changes among other features. After the signal leaves the retina, it travels through the optic chiasm via the ganglion cells axons forming the optic nerve; there, a part of these axons crosses the chiasm to the contralateral hemisphere of the brain and the remaining part continue ipsilaterally.

Figure 1. Basic connectivity of the visual system from retina to primary visual cortex for species with frontal eyes (A) such as cats, and for species with lateral eyes (B) such as agoutis.

Once the optic nerve is divided, the new set of ganglion cell axons (at this point, axons from both eyes) form a new structure called the optic tract. This tract projects to several subcortical structures like the pretectum, the Edinger-Westphal nucleus, the suprachiasmatic nucleus or the superior colliculus where the visual signals are respectively used to steer the pupillary light reflex, oculomotor functions, the night/day cycle and the coordinated movement of eyes and head. However, most of the optic
tract projects to the dorsal lateral geniculate nucleus (LGN) of the thalamus, which is a first order relay (Sherman 2007) between the retinal projections and the primary visual cortex (Fig. 1).

Neurons in primary visual cortex (striate cortex, V1, or Brodmann area 17) are embedded in a highly structured network and are activated according to basic stimulus features. The activation of a V1 neuron results from stimulating a spatially restricted area of the visual field. Light changes in different such areas activate different neurons across V1. This portion of the visual field is called the classical receptive field (CRF) of a neuron and, for the visual system in cats and monkeys, its main features are well-described by the extensive work of Hubel and Wiesel over 50 years ago (Hubel and Wiesel 1959; 1960; 1962; 1963; 1965; 1968).

The topographic specificity of the neuronal response gives rise to what is known as ‘retinotopy’, a conservation of the topology of the visual field in the visual cortex. It implies that stimulation from a particular spot of the visual field will always activate the same neurons of V1, and stimulation from neighboring regions of the visual field will (more often than not) elicit neural responses in neighboring regions of V1. As far as we know, retinotopy is conserved in all mammals.

But stimulus location is not the only stimulus feature encoded in V1. As shown by the same authors, other stimulus properties can modulate the response of neurons in this area. In experiments stimulating with moving contrast borders (i.e. bars) Hubel and Wiesel also found that a specific bar orientation, size and/or movement direction (preferred parameters) produces the optimal response (highest firing rate) for a given neuron.

1.2 Columnar organization

In all carnivores and primates studied so far, V1 neurons are arranged in such a way that neighboring neurons (belonging to the same ‘column’) share similar response properties. These ‘columns’ extend across all cortical layers and their width depends on which features they encode, the species, the anatomical position, etc. (Horton and Adams, 2005). Different types of columns can be defined based on the stimulus
feature the neuron is responding to or the thalamocortical input it is receiving, i.e. neurons in the same ocular dominance column receive predominantly input from the same eye. In cat area 17, the distance between adjacent columns of the same preference is approximately 900 μm (Schmidt and Löwel, 2002).

Another functional feature that is represented in a columnar organization is orientation selectivity. In this case, neurons in the same column have a similar stimulus orientation preference. Furthermore, the transition between columns of different angle preferences occurs gradually around singularities (pinwheel centers), and the resulting pattern of columns is referred to as an orientation preference map (OPM). Applying optical imaging techniques and color-coding the responses to several stimulus orientations it is possible to construct an OPM of a given cortical superficial area (Fig. 2).

![Figure 2. Orientation preference map resulting from intrinsic signal optical imaging in cat V1. Preferred orientation is color-coded according to the scheme on the right. [Courtesy of Dr. Kerstin Schmidt]](image)

Although these OPMs were found and described for all carnivores and primates studied so far, no such cortical organization has been found in rodents (e.g. rats, mice and squirrels, Scholl et al. 2016, Van Hooser et al. 2005 and 2007). Figure 3 shows experiments performed both in cats and rats (Okhi et al. 2005 and 2006) using 2-photon imaging demonstrating nearby single cell responses to different stimulus orientations. These experiments confirmed at a higher resolution what the optical imaging experiments had shown over a decade before, i.e. the continuous organization of cells responding to similar stimulus features in cat visual cortex. But they also showed, for the first time, the apparent lack of organization in the rat visual cortex.
Later, this proved to be the case as well for mouse visual cortex (Bonin et al., 2011). The rodent architecture was termed ‘salt and pepper’ or ‘interspersed’ and a random representation of orientation preference was assumed.

Figure 3. Single cell imaging of cat (left) and rat (right) visual cortex. Animals were visually stimulated with drifting gratings of four different orientations, while neuronal activation was measured via fluorescence imaging of intracellular calcium indicator (Oregon green). Cells are color coded following the legend in the middle, according to their orientation angle preference. Adapted from Ohki et al., 2005 and 2006.

Plenty of other species have also been investigated and, when observed from an evolutionary perspective, a pattern seems to arise (Kaschube et al., 2010, Keil et al., 2012). Figure 4 shows a representative pool of the species examined. The figure clearly shows that all rodents studied so far have an interspersed organization (Kaschube, 2014).
In the past few years several studies (Florez-Weidinger PhD thesis, 2013, Meng et al. 2012, Weigand et al. 2017), mostly theoretical, have proposed that phylogeny may not be as deterministic as the community assumed for years, suggesting that brain size, number and density of neurons may be relevant factors that influence the appearance of functional columns. The main argument behind these ideas is the high energy cost that an interspersed organization has in comparison to a columnar one. Cells that respond to similar properties tend to be interconnected (Hebb 1949), therefore, the more organized the anatomical arrangement (i.e. orientation preference maps) the closer (and faster) the information will be transmitted and the shorter the axons will be (Koulakov and Chklovskii 2001). Therefore, as brains get bigger and denser, it would be
cost efficient to organize the cells by functional similarity (Chklovskii and Koulakov 2004).

In 2016, Ringach et al. published very similar experiments to those of Okhi et al. (2005) but came to a different conclusion. They constructed a similarity index that takes into account two variables, orientation preference and spatial frequency. With this method, they compared the preferences of neurons at a certain distance, both in their newly gathered data and by re-analyzing the original Okhi figures. Surprisingly, it turned out that what years before had been said to be randomly organized, actually presents a short range spatial clustering of functional responses (distance < 50 μm). Although no OPMs or pinwheels were found, the authors could show that the similarity holds across the axis perpendicular to the cortical surface and named their finding ‘mini-columns’: a one-cell-wide vertical array of cell bodies running perpendicular to the cortical surface.

Only two months after the study from Ringach et al. (2016) was published, Kondo et al. (2016, Ohki lab) also demonstrated, through 2-photon imaging, the presence of mini-columns and described them as 30 μm long and 15 μm in diameter, radial anatomical segments of cortex, containing up to five neurons each. Although orientation preference similarity within the mini-columns was higher than expected if cells were randomly distributed along the cortex, most of them still showed a high variation of angle preference (which has no counterpart in higher mammals). With these results they argued that the mini-columnar organization is not simply a scaled down version of the orientation maps of higher mammals. And they speculate that ‘...mini-columns with diverse orientations may be assigned to represent visual information more efficiently with a small number of neurons in the small brain. The mixed functional architecture in mouse V1 may be the compromise between the brain size and keeping the vertical clusters of similarly tuned neurons at least in a subset of clusters’.

A year later, Maruoka et al. (2017) confirmed the results of Kondo et al. (2016). However, they termed the structures microcolumns ("...a group of neurons roughly located vertically, and their apical dendrites (layer 2/3 and 5 pyramidal neurons) make a bundle in the upper layers...") and limited their analyses to cortical layer 5. Furthermore, they demonstrated that orientation preference similarity within each microcolumn was dependent on the type of neuron involved. They analyzed four types
of microcolumns, two composed of excitatory and two of inhibitory neuronal types, namely: subcerebral projection neurons (SCPN), cortical projection neurons (CPN), parvalbumin-expressing cells (PV+) and somatostatin-expressing cells (SOM+). They found that, whereas the SCPN and CPN neurons do not mix when forming microcolumns, PV+ and SOM+ do mix, but only with SCPN neurons. Also, they hypothesize that orientation columns in higher mammals might be constructed on the basis of the microcolumns, because of the shared hexagonal lattice symmetry (Paik and Ringach 2012).

Moreover, in 2017, Weigand et al. published a theoretical study in which they analyzed the emergence of pinwheels and orientation maps in neural networks. They concluded that a phase transition between interspersed and orientation map organizations can occur without changing the selectivity of the connections, dependent on the amount and density of neurons participating in the network. With this result they literally say ‘The emergence of a structured map in larger rodents is, therefore, likely. The agouti is a rodent with neuronal numbers in V1 that are as high as those of other species with structured OP maps (Fig. 4A and Tables S1 and S2, [of the original paper]). Although occurrences of structured maps in the agouti and the even larger capybara have not yet been reported, they likely possess OP maps’.

Overall, the above mentioned studies show that the ‘orientation maps/interspersed’, ‘columns/no-columns’, ‘organized/random’ cortical architectures are not as black and white and straightforward divisible according to phyla as it was historically assumed to be.

The question arises: as tempting as it may be to assume that the functional organization of rodents is determined solely on a phylogenetic basis, couldn’t there be a bias in the studies given the fact that all the rodent species studied so far have been of small brain size and/or nocturnal/crepuscular habits and/or not highly visual in their behavior?

In order to examine whether the cortical architecture follows a phylogenetically defined trait, or whether it is also influenced by other variables, we studied a larger, diurnal and more visual rodent.
1.3 Visual system of Agoutis

Agouti is the common name given to several rodent species of the genus *Dasyprocta*. They are native mainly to South America, and to a lesser extent to the southern regions of Central America. The most distinctive trait among the species is the coloration of the coarse hair they present all over their bodies, which they raise when alarmed. They usually weigh 2-6 kg and are 40-76 cm long, which makes them quite similar to a domestic cat in size (Nowak 1991).

Agoutis are diurnal (Cid et al 2015) and they feed mainly gathering resources from the ground such as fruits, leaves, roots and nuts (Henry 1999). Thanks to their good handling skills and strong sharp teeth, they are one of the few species able to open *castanhas do pará* without tools, and their habitat distribution has been found to be closely related to these trees (Jorge and Peres 2005).

Their main predators are jaguars, ocelots and coatis, which they avoid when food is abundant by lowering their activity on predator peak activity times such as dawn and dusk (Suselbeek 2014). When alarmed, agoutis raise their coarse back hair and stomping their feet on the ground. When that doesn’t work, they choose flight over fight, as they are very fast runners and can jump almost two meters vertically. Only when cornered they resolve to fight, for which their main weapon is their strong bite.

Of all these characteristics, there are a few which are especially relevant for the present work. They are diurnal and can run fast and jump high, which implies they are highly visual. And they are big, very similar in size to a domestic cat, and larger than species that present orientation columns such as ferrets, tree shrews, marmosets and galagos (Fig. 4 and 5), and the rat and squirrel (biggest rodents studied so far for the presence of orientation columns).
To date, there are no records of orientation selectivity and functional anatomy experiments performed in big rodents V1 (agouti or capybara). Fortunately, there is literature on experiments in visual cortex of agoutis performed by Picanço-Diniz and collaborators at the Universidade Federal do Pará, Brazil (Picanço-Diniz et al 1991, Elston et al 2006, Picanço-Diniz et al 2011, Dias et al 2014). These experiments consisted of mainly single-electrode electrophysiology in the neocortex of anesthetized agoutis. The stimulation was performed with hand-held cards drifted tangentially to a hemisphere spanning the visual field. This way they searched for the receptive field at several positions in the cortex where they made lesions with the electrode. Subsequently, they processed the cortices histologically in order to, together with the receptive field locations, delineate the retinotopy map and determine the anatomical outline of the agouti visual cortex.

1.4 Our work

With all this in mind, we set out to search for orientation and direction selective neurons in agouti V1. Since agoutis are not a traditional animal model, we first had to set up the experimental procedures, which would render stable and reliable recording sessions.

Based on the lab’s experience performing this type of experiments with cats, and not without effort, we were able to develop an experimental protocol (see Methods) that allowed for stable recordings of anesthetized agoutis for up to 96 hours (four consecutive days). Once this was established, we were able to perform electrophysiology experiments both in agoutis and cats.
In this experimental framework, it was very valuable to do experiments in cats, in order to compare each finding we had in the agouti to the findings in the very well established cat model obtained with the same experimental protocol and stimuli.

2. Hypotheses

- The proportion of orientation and direction selective neurons in agouti is similar to that of cats.
- Agouti orientation selective neurons are not randomly distributed in V1.
- Neurons in agouti visual cortex are organized in columnar cortical maps such as in cats.

3. Objectives

- To set up the experimental procedures to obtain data from agouti visual cortex.
- To record in agouti and cat visual cortex using stimuli probing orientation selectivity.
- To reproduce results from cat literature with our methods.
- To compare agouti and cat data.
- To present evidence that either confirms or refutes a columnar map organization for orientation preference in agouti visual cortex.
4. Methods

Experiments were performed in five adult cats and eleven adult agoutis (all female). All experiments were performed at the Brain Institute, Federal University of Rio Grande do Norte (UFRN), in Natal, and approved by the Comité de Ética da UFRN (CEUA, protocol number: 006/2013). Experiments with agoutis were approved by the organization Sistema de Autorização e Informação em Biodiversidade (SISBio, protocol number: 53756). All cats were bred in the Brain Institute’s colony. Agoutis were obtained in groups of 4-6 from the Universidade Federal Rural do Semi-Árido in Mossoró (UFERSA) with the support of Prof. Moacir Franco de Oliveira, and then held on a short-term basis in the institute.

4.1 Anesthesia and physiological monitoring

Animals were prepared and maintained under anesthesia and artificially ventilated for all the experiments presented in this work. Initial anesthesia was induced via an intramuscular injection of a 20 mg/kg of ketamine (Quetamina, Vetecia LTDA, Brazil) and 2 mg/kg of xylazine hydrochloride (Virbaxyl, Rhobifarma LTDA, Brazil) cocktail for cats. Agoutis showed a higher resistance to this initial cocktail of anesthesia, therefore the dosage used to successfully induce anesthesia in these rodents was 40 mg/kg of ketamine and 10 mg/kg of xylazine. Subsequently, a tracheostomy was performed and a cannula inserted in the trachea for artificial ventilation. In order to prevent inflammation, a daily dose of 0.1 mg/kg of dexamethasone (Cortiflan, Ourofino, Brazil) was administered intravenously.

A mixture of O₂/N₂O (25/75%) was pumped through a halothane (for all cats and two agoutis) or isoflurane (for the rest of the agoutis) vaporizer to respirate the animal through the tracheostomy. The vaporized anesthetic concentration was maintained between 1-2% (for isoflurane) and 1-1.5% (for halothane) throughout the surgery, and lowered to 0.8 % (for isoflurane) and 0.6% (for halothane) during the recordings.

Artificial ventilation was performed via a hydraulic pump (Harvard Apparatus) throughout the experiments. For cats, tidal volume was in the 40-50 ml range and
respiration frequency 18-22 breaths per minute. For agoutis, tidal volume was in the 30-35 ml range and respiration frequency 25-30 breaths per minute.

In order to control for involuntary eye movements, after completion of surgical procedures, and throughout the experiment, the animals were paralyzed by an initial intravenous push of 0.6 mg/kg for cats and 0.3 mg/kg for agoutis of pancuronium bromide (Pancuron, Cristália, Brazil), followed by continuous intravenous infusion of 0.15 mg/kg/hr for cats and 0.10 mg/kg/hr for agoutis of the same drug.

The physiological stability of the animals under anesthesia was evaluated by continuously monitoring the body temperature, inspiratory pressure, electrocardiogram and CO$_2$ levels in the expired air. During all experiments, the heart rate remained between 90-220 bpm, and we aimed at a CO$_2$ output in the range of 2.5-3.8%.

4.2 Electrode placement

Two types of electrodes were used for all experiments described here, although never combining the different types within the same individual animal. From here on, we will call the two types of electrodes arrays or probes.

‘Arrays’ are 4 x 4 arrays of platin-iridium electrodes insulated by epoxy coating (1MΩ, 50 µm diameter, Microprobes, Gaithersburg, MA, USA). These arrays have an inter-electrode spacing of 250 or 400 µm, depending on the experiment. ‘Probes’ have two shanks of 16 recording sites each, arranged vertically (NeuroNexus, Ann Arbor, MI, USA). The spacing between shanks is 500 µm, and 100 µm between adjacent recording sites.

For arrays, either one, two or three (16, 32 or 48 electrodes) were inserted simultaneously in each experiment, in the visual cortex of one brain hemisphere. When probes were used, only one (32 recording sites) was inserted (Fig. 6).

In cats, electrode implantation was aimed at area 17, area 18 and the transition zone between them, according to stereotaxic coordinates and/or anatomical landmarks. In agoutis, electrodes were aimed at V1 according to the visual cortex description.
available (Picanço-Diniz 1991, Dias et al. 2014). After electrode insertion and confirmation of visual responses, agarose solution (3-5% in 0.9% NaCl) was placed over the craniotomy to prevent desiccation and reduce pulsations.

Figure 6. Photos of electrode placement. (Left) Two array electrodes. (Right) Probe electrodes.

4.3 Visual stimulation

Eyes were treated with drops of atropine 1% (Allergan Inc., Brazil) and neosynephrine 1% (Ursapharm, Germany), in order to dilate the pupils and retract the nictitating membrane. Eye refraction was measured with a Rodenstock refractometer and appropriate correcting contact lenses with an artificial pupil of 3 mm were applied in order to refract the optical apparatus to -1.5 diopters approximately. For cats, lenses in the range of (-1) to (+1) diopters, with a diameter of 15 mm and a radius of 8.5 mm were used. For agoutis, lenses in the range of 7 to 9 diopters, with a diameter of 10 mm and a radius of 6.55 mm were used. The large diopters correction needed for agoutis is most likely due to contact lens shape not adjusting perfectly to agouti eye curvature.
Figure 7. Stimulation setup scheme. Both animal head and monitor positions were independently adjustable. Red point represents the hinge of a bolt held to the animal's head with dental acrylic. Hinge bolt was secured to stereotactic device.

Stimuli were presented on a 21” CRT monitor placed at 57 cm in front of the animals’ eyes (Fig. 7). This distance was selected so that 1 cm on the monitor screen corresponded to 1 degree of visual field angle. This rendered a stimulation area of 30x40 degrees of the visual field.

Two general types of stimulation were presented during the experiments, namely: contrast reversing checkerboards and drifting gratings. Contrast reversing checkerboards consisted of a black and white checkerboard of seven different square sizes (spatial frequencies 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 and 2.56 cyc/deg) reversing at three different temporal frequencies (2, 4 and 8 Hz). Sine wave gratings were presented in 12 different directions of movement (intervals of 30 degrees) and two temporal frequencies (2 and 4 Hz).
4.4 Data acquisition

Before data recording, the recording sites’ CRFs were identified based on stimulation with a hand-held slit lamp. The monitor was positioned by means of a moving support, parallel to the recorded eye(s) and maximizing the number of CRFs within the screen’s limits.

Cats were stimulated binocularly. Agoutis, due to their laterally positioned eyes, were stimulated monocularly. Stimulation eccentricity was determined by measuring angle between the position of the recorded response and an imaginary line through the middle of the animal’s nose (visual field vertical meridian).

Both recording and stimulus presentation were controlled using custom software in LabView (MEC and SPASS by S. Neuenschwander). In a first step, a CRF mapping was performed by stimulating with a single bar oriented in 22.5° steps moving in the perpendicular direction (16 conditions). For each condition, a peri-stimulus time histogram (PSTH) of the spiking response to the drifting bar was calculated (width = 1°, length = 30°, speed = 20°/s) using a Gaussian smoothing kernel with a SD of 12.7 ms for each of the 16 mapping directions. Then, each PSTH was normalized to its maximum height in order to weight each stimulus direction equally. Subsequently, PSTHs for a certain channel were projected to the visual mapping field and summed across stimulus directions. The resulting response density maps were additionally low pass filtered (2D Gaussian smoothing with a SD of 5.88 deg) and the receptive field was defined as the area above 70% of the maximal response (Fiorani et al., 2014; Conde-Ocazionez et al., 2017).

Then, recording sessions were performed. We used a Plexon pre-amplifier (Plexon Inc., Dallas, TX, USA) to amplify (1000 fold) and high pass filter (0.7 – 6 kHz, for MUA) or low pass filter (1 - 100 Hz, for local field potentials) the signals. Local field potential (LFP) and spiking multiunit activity from all recording sites were collected at 1 KHz and 32 KHz respectively. Spiking events were detected by voltage threshold crossing. Single units were identified using the WaveClus spike-sorting algorithm (Quian-Quiroga, 2004). WaveClus is an automatic spike-sorting method based on wavelet decomposition and superparamagnetic clustering. The spike sorting is especially relevant in the present work, because it allows us to compare neurons that were
recorded within the same electrode, which would be otherwise impossible. It is important to note that although, in the present work, single units resulting from the spike sorting will be interpreted as neurons. Therefore the terms ‘single-unit’ and ‘neuron’ will be considered synonyms in the scope of our own results.

Each trial consisted of 500 ms of pre-stimulus activity recording while an isoluminant grey screen was presented on the monitor screen, followed by 2000 ms of stimulus presentation (Fig. 8). Within each stimulation protocol, each stimulus condition was repeated at least 15 times in a pseudo-randomized order.

Figure 8. Upper panel: Time course of the stimulation during a trial. Lower panel: Sketches of the two types of stimuli used. Checkerboards (left) have no direction/orientation component, consisting solely of black and white contrast-reversing checkers. Gratings (right) drifted in one of twelve possible directions.
4.5 Data Analysis

All data were processed using Matlab (R2014b, Mathworks) custom made codes to read from LabView (National Instruments), organize, select and analyze the recorded data.

4.5.1 Visual evoked potentials

Visual evoked potentials were computed from the LFP recordings for the contrast-reversing checkerboard stimulation. The VEP is the average of the LFPs for all trials of the same condition, and it is characterized by its amplitude and peak latency.

In order to characterize the response profile of each recording site to the checkerboard stimulus we developed a classifier algorithm to automatically determine if the recording came from an electrode implanted in A17, A18 or the transition zone (TZ) of the cat visual cortex. We applied this algorithm to the agouti data from V1 to compare the classification with that of the cat visual areas.

The algorithm was set up using a subset of recordings from two cats. The recordings used for this were the response profile for the checkerboard stimulation protocol (VEP amplitude for each of the 21 conditions: 7 spatial frequencies and 3 temporal frequencies). The setup consisted of obtaining the first three principal components of that set of data and projecting those response profiles onto the PCA space by performing a dot product between each response profile and the eigenvectors obtained in the setup. With that 3D projection, a \textit{k-means} clustering was performed. We demanded three clusters from the \textit{k-means}, because we knew that electrodes were placed in up to three different functional areas during the experiments, namely: A18, A17 and TZ. After the clustering, we calculated the centroid of each cluster and assigned each centroid to one of the three areas. The assignment was done by visual comparison of the spatial frequency tuning curves of the clusters’ with the real tuning curves of the areas, thoroughly described in the literature (Tolhurst, D. J., and I. D. Thompson 1981, Skottun et al 1986, Issa et al 2000, Ribot et al 2013, Zhao et al 2016), briefly: A18 exhibits stronger responses to low spatial frequencies, A17 to high spatial frequencies, and the TZ a more stable profile across the spatial frequencies investigated here.
We then projected all the responses recorded onto the PCA space and assigned cluster identity to each of them. The responses that fell further away than the median + 1.5 median absolute deviations from the centroid of the closest cluster were discarded.

### 4.5.2 Firing rates

For each stimulus condition, the mean firing rate (FR), defined by equation 1 was computed for each recording site as a response to each stimulus condition

$$ FR(c) = \frac{\sum_{i} spk(tr_c)|_{t_0}^{t_1}}{t_1-t_0} \quad \text{eq. 1} $$

where \( FR(c) \) denotes the mean firing rate during the presentation of stimulus condition \( c \), computed by adding the spike counts \( spk \) obtained in each trial \( tr \) in the corresponding condition \( c \) and dividing the sum by the corresponding time interval. For example, when computing the evoked FR for a grating condition, \( t_1=2500 \) and \( t_0=500 \).

### 4.5.3 Orientation and Direction Tuning

For the drifting gratings, we characterized the multi and single-unit activity from each recording site according to three main parameters: increase of activity between pre-stimulus (‘blank’) and stimulus recordings, orientation preference, and direction of motion preference.

Increase of activity was defined by a significant sign test result of comparing the firing rates recorded during the 500 ms ‘blank’ period and during the stimulation period.

Orientation and direction preference were defined by an angle and a tuning index (TI). The tuning index was calculated by equation 2, i.e. by vectorial addition of spike counts across all trials and stimulus orientations or directions,
where Tuning Index can be an orientation selectivity index (OSI) or a direction selectivity index (DSI), \( R(\theta) \) is a vector with magnitude equal to the accumulated spike count as response to condition \( I \) across all repetitions and angle defined by \( \theta \). The TI has a range between 0 and 1 considering the former as no tuning, where the unit has exactly the same response (if any) to all conditions and the latter as the perfectly selective tuning where the unit responds only to a single condition. The angle of the TI resulting vector corresponds to the preferred angle of the unit.

For direction tuning calculations, the twelve directions of movement present in our drifting grating stimuli were used. For orientation tuning calculations, the responses to parallel directions of movement were summed (e.g. response to 0 deg + response to 180 deg), resulting in six stimulus orientations.

### 4.5.4 Tuning index threshold

In previous cat experiments (Wunderle et al 2013, Peiker et al., 2013), a fixed threshold of 0.2 was used for the tuning index to define the subsample of selective units (be it for orientation or direction, equivalently) considered for further analysis. This value serves well for recordings in cats. As other authors have noted before (Mazurek et al 2014), this threshold might be inappropriate when studying species/units that present different properties (such as low firing rate) as compared to the ones traditionally studied (i.e., cat V1 neurons).

Agouti recordings present lower firing rates than cats, making it more likely to get a high tuning index simply by sporadic spontaneous activity. This becomes clear when imagining the example of a neuron firing only a few action potentials (APs) over a few trials, and remaining silent during the rest of the stimulation protocol. If this neuron were a selective unit, all APs should appear on trials of the preferred orientation. But if it was only spontaneous activity that we are seeing, then, the fewer the APs, the higher the chance of all of them appearing in trials of the same orientation/condition. Therefore, the fewer the spikes recorded (lower FR), the higher the chance of a non-
selective cell presenting a spurious high tuning index and therefore being characterized as a selective one (false positive).

In order to minimize false positives we applied a simple method to adjust the threshold depending on the amount of APs fired by the unit in question over the stimulation protocol. For each amount of APs occurring in a stimulation protocol (2 to 4000), we simulated 10000 TIs resulting from random AP firing (non-selective units). Figure 9 shows the histogram of TIs simulation. As can be noted in the distribution, even though the spikes simulated were random, a lot of TIs are well above the traditional 0.2 threshold. For our data, we decided to use a moving threshold that depends on the number of spikes fired by the unit in question. Such threshold will be the 95th percentile of the histogram distribution of simulated TIs for each particular number of spikes. Figure 9 shows the resulting threshold curve of the 95th percentile from all these simulations.

Figure 9. Left: Histogram of TIs for 10000 simulations of randomly distributed spikes. Black vertical dotted lines depict the 95th percentile threshold for each distribution. Right: 95th percentile TI threshold versus number of spikes. Black circles depict the thresholds for the simulations in A.
4.5.5 Orientation and direction tuning curves

In order to compare neuronal orientation and direction selectivity between species and areas, and with previous literature, tuning curves and their corresponding half width at half height (HWHH) were calculated. For every orientation/direction selective neuron, the normalized firing rate (relative to its own maximum firing rate at the optimal stimulus orientation/direction) was calculated, resulting in an orientation/direction response profile for each selective neuron. Then, the response profiles were centered at the optimal response and a Gaussian curve was fitted to the each response profile and a HWHH value with its corresponding error was calculated for the appropriate subpopulation of neurons (orientation/direction, tuning index threshold, species, and spatial frequency).

4.5.6 Cortical distance calculations

In order to compare neurons across the cortex, we calculated the distance between recording sites. This was done by calculating the distances between all array electrodes in the 250 μm spacing arrays. Neurons recorded within the same electrode were considered to be 0 μm apart, neurons in adjacent electrodes were assumed to be either 250 μm or 353 μm (for the closest diagonal), and so on. This way, the longest distance we were able to compare was 1060 μm, which corresponds to the distance between electrodes located at opposite vertices of the array.

It is important to note that we have no way of determining the exact distance between recorded neurons. We can only be sure that neurons spike-sorted from the same electrode are closer together than neurons recorded in adjacent electrodes, and so on. Similarly, the cortical distance between electrodes in each dataset is not necessarily the calculated one, mainly because of electrode bending during implantation. Therefore, all the declared distances of neuronal comparisons should be taken as rough estimates of neuronal cortical distance.
4.5.7 Orientation preference similarity measures

We implemented two different measures of similarity for orientation selective neurons: (i) the absolute angle difference and (ii) the similarity index based on the correlation of tuning curves.

(i) For every orientation selective neuron (or multi-unit) an angular preference was calculated (Eq. 2). The first measure of similarity between units was therefore the difference in the preferred angle. In the orientation space, where preferred angles can only range between 0 and 180 deg, this measure goes from 0 deg (for units with the same preferred orientation angle) to 90 deg (for units tuned to orthogonal stimuli).

(ii) The firing rate of each orientation selective unit was calculated for each of the 6 stimulation orientations (summing parallel conditions of the 12 directions of movement, Fig. 8). Then, for every pairwise comparison of units, the Pearson correlation was calculated between the firing rates profile (the orientation tuning curve for each unit). This measure ranges from 1 (for units with extremely similar orientation tuning curves) to -1 (for units tuned with orthogonal tuning curves).

When analyzing the statistical significance of these measures, the obtained data points were compared to the same analysis, but after shuffling distance and angle data. We termed this shuffled results ‘surrogate’. Comparisons were then made for each data pair (real versus surrogate) using Mann-Whitney tests.
5. Results

We detected and recorded orientation and direction selective neurons, and characterized the response parameters and functional anatomy of agoutis. In particular, we characterized the optimal stimulation parameters (spatial and temporal frequencies), size of receptive fields, functional orientation and direction tuning curves and selectivity indices, functional similarity across horizontal and vertical dimensions of the cortex. The results will be laid out in detail in the following sections.

5.1 Agouti experimental setup

Agoutis were not docile and friendly upon arrival at the Brain Institute (ICe), showing signs of stress (stomping feet and raised back hair) whenever someone approached their enclosure. After two weeks of acclimatization at ICe, the animals adapted to the new habitat, to the point where the animals would approach investigators that offered food, although always ‘keeping an eye’ on them.

This expression represents the visual behavior of these animals very well. Even though agoutis possess a binocular visual field of approximately 20 degrees in width (Picanço-Diniz et al. 2011), they prefer to follow the movements only with one eye, positioning themselves showing only one profile, and keeping one (and only one) eye on the target.

Once animals were accustomed at ICe, we started performing our experiments. Since agoutis are very similar in size to cats, our initial approach was to perform experiments just as we had been successfully performing them with cats. Soon it became clear that some devices and procedures needed to be adapted.

Regarding induction of anesthesia, we were instructed by our collaborators at UFERSA that agoutis need high amounts of intramuscular ketamine/xylazine in order to reach good levels of anesthesia. Even though we started with ketamine doses similar to those of cats, we soon found it necessary to increment the dosage. All agoutis received at least double the ketamine (and as much as six times in one particular experiment), and five times as much xylazine than cats. We never saw any
complications arising from such doses. We hypothesize that we needed increased doses for two reasons, namely: the high metabolism of rodents, especially under stress circumstances (when being caught from the enclosure for initial anesthesia), and/or possible previous exposure of these agouti individuals to ketamine/xylazine. Our Mossoró collaborators’ research involves dosing of hormone levels across time, so most of the individuals had been previously subjected to weekly doses for blood sample drawing, which might have built some resistance in these animals.

Intravenous access was not possible via a butterfly catheter (as with cats), due to the agoutis’ small forearm vein caliber and difficult access of the hind leg veins during the visual experiment. Therefore, we had to perform a microsurgery (venae sectio), dissecting a forearm vein and introducing a custom-made flexible catheter (diameter < 0.5 mm).

For maintaining anesthesia, we initially chose halothane as inhalation anesthetic because it had been previously observed in cats that imaging experiments run more stable with halothane than with isoflurane because of lesser arrhythmicity (Wunderle, Schmidt, personal communication). Although there was previous literature stating that halothane is a viable anesthetic for the agouti (Bacher et al, 1976), we found problems maintaining agoutis under halothane anesthesia for long periods of time. After a few hours of halothane respiration, the animals presented a marked bradycardia, which we treated with the sympathomimetic drug orciprenaline intravenously (0.05ml of orciprenaline 0.5mg/ml, Alupent, Boehringer Ingelheim). Alongside with the bradycardia, we also noted a swelling of the abdominal cavity compressing the thoracic cavity, which made ventilation increasingly difficult. Out of six agoutis, which were held under halothane anesthesia, only two survived long enough (more than 12 hours) for us to obtain a sufficient amount of electrophysiological signals.

Due to these problems, we decided to switch to isoflurane anesthesia and to put the thermometer externally between the belly and the heating pad (rather than rectally, in order to avoid any sphincter clogging and internal gas buildup). After introducing these modifications we were able to maintain the animals under stable anesthesia and record for several days.
Table 1 shows the summary of experiments performed. All cats came from the ICe colony. All of the 16 agoutis were brought from Mossoró and successfully maintained in captivity at ICe for up to six months.

<table>
<thead>
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<th>Animal code</th>
<th>Species</th>
<th>Anesthesia</th>
<th>Data Collected</th>
</tr>
</thead>
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<td>Cat</td>
<td>x</td>
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<td>Cat</td>
<td>x</td>
<td>250 μm 2 48 Left 82</td>
</tr>
<tr>
<td>c39</td>
<td>Cat</td>
<td>x</td>
<td>250 μm 3 48 Left 98</td>
</tr>
<tr>
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<td>Cat</td>
<td>x</td>
<td>250 μm 1 48 Left 51</td>
</tr>
<tr>
<td>c41</td>
<td>Cat</td>
<td>x</td>
<td>250 μm 2 48 Left 58</td>
</tr>
<tr>
<td>- Agouti</td>
<td>x</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>- Agouti</td>
<td>x</td>
<td>N/A</td>
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<td>- Agouti</td>
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</tr>
<tr>
<td>- Agouti</td>
<td>x</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>c36</td>
<td>Agouti</td>
<td>x</td>
<td>250 μm 3 48 Left 98</td>
</tr>
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<td>x</td>
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<td>x</td>
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<td>Agouti</td>
<td>x</td>
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<td>Agouti</td>
<td>x</td>
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<td>x</td>
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<td>x</td>
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<tr>
<td>c52</td>
<td>Agouti</td>
<td>x</td>
<td>250 μm 5 16 Both (not simultaneously) 81</td>
</tr>
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</table>

Table 1. Summary of experiments performed. Stimulation was always binocular for cats and monocular for agoutis. Agoutis’ stimulated eye was always contralateral to hemisphere recorded.
5.2 Functional properties of cat and agouti visual cortex units

5.2.1 Receptive field size

Before recording every dataset, classical receptive fields (CRFs) were mapped (see Methods) for each electrode (multi-unit). In cats, we were able to stimulate binocularity after aligning the eyes using an optic prism. Due to the cats’ frontally positioned eyes recordings were performed closer to the visual field midline than agouti’s. In agoutis, due to their laterally positioned eyes, we stimulated monocularly, with the monitor positioned away from the midline. This is why there is little overlap in the recorded CRFs eccentricity of the two species.

Figure 10. A: Receptive field size as a function of eccentricity. B: CRF mapping examples in cat area 17 (azimuth and elevation less than 5°), and agouti V1 (azimuth approximately 28°, elevation less than 5°). Scale bars = 2°. Data from 4 cats and 11 agoutis; 125 multi-units for cat A18, 82 for cat A17, and 401 for agouti V1.

Figure 10 shows two CRFs and a quantification of receptive field size as a function of visual field eccentricity. All the units recorded had RFs close to the horizontal meridian of the visual field (±15 degrees in latitude).
Our measurements for agouti CRF size are in the 1-22 deg\(^2\) range. Cat A18 CRF sizes are in the 1-32 deg\(^2\) range, and A17 CRF sizes are in the 0.5-22 deg\(^2\) range. Agouti CRFs size distribution is much more similar to Cat A17 than Cat (Figure 11).

Area 18 is known to have bigger CRFs (2-32 deg\(^2\) range reported in Hubel & Wiesel 1965) and selectivity to broader spatial frequencies than area 17. Characteristically for area 18, it can be observed that the cat’s CRF size increases rapidly with eccentricity while the agoutis’ CRFs remain small at large eccentricities of up to 120 degrees.

Figure 11. A: Histograms of receptive fields size. B: Boxplots of cat and agouti RF sizes.

5.2.2 Visual evoked potential amplitudes

Contrast reversing checkerboards of 3 temporal frequencies (TF, 2, 4 and 8Hz) and 7 spatial frequencies (SF, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 and 2.56 cyc/deg) were used to determine the optimal stimulation SF and TF. LFP traces were recorded and the mean of each stimulus condition was calculated for each channel, in order to obtain the visual evoked potential (VEP) amplitude response profile (Fig. 12A).
Figure 12. Cat visual evoked potentials. A: Color-coded map of the VEP response profile amplitude separated by condition and recording (left, two cats). Conditions 1 through 7 correspond to the 7 SFs analyzed at 2Hz; 8 through 14 to the same SFs at 4Hz; and 15 through 21 at 8Hz. VEP amplitude profile for the automatic clustering (right). B: Average spatial frequency tuning curves of the VEP amplitude for each cluster of responses. Each plot corresponds to one cluster. Note the characteristic amplitude decay at 0.32 cyc/deg for area 18. Data from 2 cats.

VEP response profiles were grouped into three clusters (automatic k-means) because electrodes were inserted into three functionally different regions (A17, A18 and the transition zone (TZ) between them). We obtained tuning curves for the VEP amplitudes for each of the clusters and assigned them an identity based on their shape (Methods).

The response profile was also calculated for agouti recordings, although this time there was no clustering needed, because were implanted in the same functional area: V1.
Figure 13. A: Response profile PCA projections. Cat (open circles) data classified into TZ, A17 and A18. Agouti (filled circles) data was fed into the same classifier for comparison. Black crosses depict the clusters’ centroids. Note that the classifier assigns the majority of agouti responses to the cat area 18 cluster, with a few of them falling within the TZ cluster. B: Spatial frequency tuning curves for the agouti VEP responses. Data from 2 cats and 3 agoutis. 72% of agouti responses classified as cat A18, 27% as cat TZ, and 1% as cat A17.

Agouti responses were projected onto the same PCA space as cats’, in order to obtain an automatic measure of response similarity to cat areas. Figure 13A shows the PCA projection of cat and agouti responses, and the majority of the agouti responses (red symbols) fall within the cat A18 cluster (blue squares), and the rest within the TZ cluster. Visual inspection of agouti VEP-SF tuning curves (Figure 13B) compared to cats’ confirms the PCA classification, because of the similarity of the agouti response profile with that of cat A18.

Both spatial frequency tuning curves for cats and agoutis show that 2 Hz stimulation elicits the strongest (and 8Hz the weakest) response of all the TFs tested. We noticed that 1.28 and 2.56 cyc/deg stimulation elicited the weakest responses of all the SFs tested, with the only exception for the A17 responses to high SFs. Given the fact that most of our recording from cats were in A18, and that A17 shows a preference to 0.32 and 0.64 cyc/deg stimuli, all further analyses were restricted to SFs lower or equal to 0.64 cyc/deg.
5.2.3 Orientation and Direction Selectivity

Drifting sine wave gratings were used to study orientation and direction selective responses. Since orientation/direction selectivity were not studied in agoutis before, no optimal stimulation parameters were at hand. Thus, we focused on the selectivity of neurons to the five spatial and two temporal frequencies we found best responses for in the VEP analysis.

Figure 14. Representative agouti multi-unit. A: Classical receptive field (CRF) mapped by moving bars. Color codes for firing rate according to stimulus position. CRF area is 10deg², azimuth approximately 20°, elevation less than 5°. Scale bar = 5°. B: Polar histogram of each direction of grating movement (SF = 0.16 cyc/deg, TF = 2 Hz, OSI = 0.23). C: PSTH of the trials corresponding to the 180° stimulation in C: Red line depicts stimulus onset. D: Orientation selectivity as a function of spatial frequency separated for the two temporal frequencies. Note that, for that unit, the selectivity peaks at 0.08 cyc/deg for both temporal frequencies.

Figure 14A shows the receptive field plot of an example of an orientation selective unit in agouti primary visual cortex. This particular unit is tuned to vertical (90 deg) contours moving in the 0/180 deg directions, as can be seen from the polar histogram.
(Figure 14B). In Figure 14C, we plotted the peri-stimulus time histogram of that unit’s firing rate from all trials to 180 degree stimulation (TF = 2 Hz, SF = 0.16 cyc/deg). This demonstrates a typical response of a selective unit, showing most of the increased firing rate to the preferred stimulus early after stimulus presentation (transient) rather than later in the trial. Noteworthy, the unit’s orientation selectivity index is dependent on both the spatial and temporal frequency of the stimulus, with highest values at 0.08 cyc/deg and 0.16 cyc/deg for 2 Hz stimulation and at 0.08 cyc/deg for 4 Hz stimulation (Figure 14D). The relationship between orientation selectivity and spatial or temporal frequency will be examined next.

5.2.4 Spatial frequency selectivity

The example unit in Figure 14 showed that the degree of orientation selectivity, as indicated by the orientation selectivity index, can depend on the spatial and temporal frequency of the stimulus. In Figure 15, we depict the population result to Figure 14D, the average orientation selectivity index as a function of the stimulus spatial and temporal frequency. In order to do so, we included only units which crossed the dynamic orientation selectivity threshold (see methods). The average spatial frequency tuning curves confirm the tendency we observed above for the agouti example unit and in the VEP analyses, namely that orientation selectivity is higher at 2 Hz stimulation and spatial frequencies between 0.04 and 0.32 cyc/deg. This holds for both cat area 18 and agouti V1. As expected, 0.64 cyc/deg stimulation produced highly selective response only for cat area 17. We assume that the surprisingly high selectivity at this spatial frequency for agouti at 4 Hz (grey color, Figure 15) as an outlier in the context of the rest of the data, and most likely a spurious result. We observed the same upwards trend for stimulation with 1.28 and 2.56 cyc/deg (data not shown), spatial frequencies which appear to be outside the cutoff frequencies deduced from the VEP responses. Thus, we interpret this finding as an artifact produced by our visual stimulation. It is conceivable that at 4 Hz the stimulation reaches a speed that the duty cycle for high spatial frequencies appears to be closer to 100% (instead of the real 50%). This might lead to fusing of bars of the gratings possibly lowering the effectively stimulated spatial frequency.
Figure 15. Spatial frequency selectivity of orientation selectivity indices for cat A17 (A), cat A18 (B) and Agouti V1 (C). Curves from 2Hz stimulation from A, B and C are plotted in the same axis in D. Only neurons that passed the OSI threshold were included. Note that cat neurons are much more orientation selective. All animals from Table 1 were included in this analysis. Data from 3 cats and 9 agoutis.

The shape of the cat spatial frequency tuning curves (Figure 15D) is as expected and similar to the one obtained in the VEP analysis (see Figure 12). Although, absolute orientation selectivity is much lower for agouti units, the shapes of their spatial frequency tuning curves (Figure 15C) have similarities to those of the cat A18, confirming the interpretation of the VEP results that agouti V1 SF preference resembles cat A18.

This result suggested that selective neurons should be more frequent at the lower spatial frequencies tested. Thus, we examined how the proportions of orientation and
direction selective units change with spatial frequency (Figure 16). As expected from the selectivity indices the ratio of units passing the dynamic selectivity threshold versus all visually responsive units in cat A18 starts to decay clearly at 0.32 cyc/deg (Figure 16, red lines).

In the agouti, differently from cat A18, the proportion of orientation or direction selective units decays more slowly. Selective neurons seem to be more evenly distributed across all tested spatial frequencies (Figure 16, yellow lines).

The proportion of orientation selective neurons detected for cat A17 - applying the dynamic threshold - is lower than expected from literature and from the comparison with A18 values. We hypothesize this may be due to our undersampling of A17 (given that most of our electrodes were in A18) and to the spatial frequencies investigated, which are better to elicit responses from A18 than from A17.

Figure 16. Left: Proportion of orientation selective (OS) neurons passing the dynamic threshold. Right: Proportion of direction selective (DS) neurons passing the dynamic threshold. Data from 2 cats and 9 agoutis.
In conclusion from the above analyses of the basic response properties of agouti visual cortical neurons, we focused all of our following analyses on 2 Hz stimulation and on SFs equal or lower than 0.64 cyc/deg.

**5.2.5 Orientation and direction tuning curves**

In the previous sections, we observed that agouti neurons exhibit much lower selectivity indices than cat neurons. Besides the selectivity index, another parameter characterizing the selectivity of neurons can be extracted from their tuning curves, namely the curves’ half-width-at-half-height (HWHH). This parameter has the advantage that it allows comparisons with published data from the only other large visual rodent studied so far (Van Hooser et al. 2005, only orientation selectivity was reported).

In order to extract the HWHH, we fitted Gaussian curves to the OS and DS normalized firing rate response profiles (see methods, Figure 17). A fixed threshold of 0.2 was chosen for the tuning index in this analysis, in order to compare only the most selective cells of both species. For both, orientation (Figure 17, A) and direction tuning curves (Figure 17, B), the HWHH values are larger for agouti units than for cat units, which implies broader tuning curves.

![Figure 17. A: Examples of gaussian fits for mean orientation tuning curves elicited at 0.32 cyc/deg of stimulation. Half width at half height (HWHH) of orientation (B) and direction (C) selectivity tuning curves. Firing rates for each stimulation orientation/direction were normalized for every neuron with a selectivity index > 0.2. A Gaussian function was fitted to each of the normalized firing rates for orientation/direction, for each spatial frequency. Note that tuning curves of cat area 18 neurons have...](image-url)
much lower HWHH values than agouti, especially for direction selectivity. Data from 2 cats and 9 agoutis.

The discrepancy between HWHH values between the two species is particularly high for direction selectivity. As expected for area 18, direction tuning is very good for these neurons, and thus the direction tuning curves are very narrow.

Agouti orientation HWHH applying a criteria of an OSI of 0.2 is in accordance with what was reported for the gray squirrel (< 70 deg, Van Hooser et al. 2005). Noteworthy, in the cited study, a more rigid criteria (OSI greater than 0.5) was applied to sample the orientation selective units from which the HWHH was calculated.

Therefore, we calculated the HWHH in our data also for cells with a OSI > 0.5 (Figure 18). With such a high threshold, the agouti HWHH for orientation tuning is lower than 23 deg for all of the five SFs (similar results for cats, data not shown). In conclusion, the highly selective agouti neurons seem to have sharper orientation tuning than the gray squirrel neurons.

Figure 18. Median (and SEM) HWHH for agouti neurons with OSI > 0.5. Dotted line depicts the median HWHH reported for Gray squirrel V1 (Van Hooser et al, 2005). Firing rates for each stimulation orientation/direction were normalized for every neuron with a selectivity index > 0.5. A Gaussian
function was fitted to the mean of the normalized firing rates for each stimulation orientation/direction, for each spatial frequency. Data from 9 agoutis.

5.2.6 Orientation and direction preference distributions

All visual cortices studied so far reveal an anisotropic distribution of angle preferences. These biases have been proposed to reflect natural image statistics (Baddeley and Hancock 1991, Sengpiel et al. 1999). For cats and monkeys, a cardinal (vertical and horizontal) bias has been manifold observed in cortical structures such as described in the response strength of V1 neurons (cats: Pettigrew et al. 1968; Frégnac & Imbert 1978; Orban et al. 1981; Leventhal & Schall 1983; monkeys: Mansfield & Ronner 1978; Blakemore et al. 1981). Since rodents have lateral eyes and usually different habitats and ecological niches, different distributions of angle preferences in our two samples of cat and agouti angle preferences are to be expected.

In Figure 19 we plotted, for every SF studied, the amount of cells which prefer either horizontal (-30 to 30 deg contours), vertical (60 to 120 deg contours) or oblique (30 to 60 and 120 to 150 deg contours) stimuli. For both species, the angle preferences of orientation selective neurons are not uniformly distributed. While cats exhibit the previously described preferences biased towards the two cardinal orientations, agoutis show a preference for horizontal contours (Figure 19).

Figure 19. Orientation preference distribution. Note that cat’s preference is biased towards both horizontal and vertical borders, while agouti’s is biased towards horizontal borders. Figure legend: colored scheme representation of horizontal (-30 to 30 deg contours), vertical (60 to 120 deg contours) or oblique (30 to 60 and 120 to 150 deg contours) stimuli. Data from 2 cats and 9 agoutis.
Also for the direction of motion preference, different anisotropic distributions have been previously described for cat area 18 (Ribot et al, 2008) and extrastriate visual areas in rodents (ground squirrel PMLS: Paolini and Sereno, 1998).

We calculated the histograms of angle preference for all DS cells recorded, for both cat and agouti samples. When describing direction angle preference, it is important to differentiate between the visual hemi-fields being analyzed. A vertical contour that moves left or right will have a different meaning depending on which hemi-field it is seen. For example, a vertical contour moving to the right, seen in the left hemi-field, corresponds to an object approaching the animal perceiving it. Analogously, the same vertical contour moving to the right, seen in the right hemi-field, corresponds to an object moving away from the animal perceiving it. Because of this, our direction bias analyses are separated by the hemi-field recorded (contralateral to the hemisphere recorded).

Figure 20A shows the number of neurons with direction preferences falling into one of four quadrants (315 to 45 deg, 45 to 135 deg, 135 to 225 deg, 225 to 315 deg). In B, the neuron numbers are depicted in normalized polar histograms binned in 10 degree compartments. Here, 0 and 180 deg are vertical contours moving to the right or left respectively, 90 and 270 deg are horizontal contours moving upwards or downwards respectively, in the visual field.

Based on the neuron numbers in each of the 36 direction bins a resulting vector was calculated by vector summation in a similar manner as expressed in equation 2. This vector is indicated by a red line in the polar histograms. It lets us conclude that, both in cats and agoutis, we found a bias in the DS cells’ preference towards directions moving away from the vertical meridian and downwards in the visual field. To test for uniformity we computed the Rayleigh uniformity test for each polar histogram. In all three cases the uniformity was rejected (p-values < 0.001).
Figure 20. Direction preference distributions. A: Histogram of the neurons' preference by quadrant of direction of movement preference and by stimulus spatial frequency. Colored legend: right (315 to 45 deg) upwards (45 to 135 deg), left (135 to 225 deg), downwards (270 to 315 deg) direction of stimulus movement. B: Polar histograms of neuron motion direction preference for all spatial frequencies, for left or right hemi-field of the visual space. Red lines depict the resulting vector of the histogram (same procedure as tuning index and angle calculation for each unit). All polar plots do exhibit a non-uniform distribution (Rayleigh uniformity test p-values: 4.24e-06, 2.57e-04, 9.09e-20 respectively). Data from 2 cats and 9 agoutis.
5.3 Spatial arrangement of orientation selective neurons

It is well known that OS neurons in the cat’s visual cortex are organized in an orientation preference map (OPM) (see Figure 2). In this type of functional organization, neurons positioned close to the same axis (perpendicular to the cortical surface) have very similar angle preferences across cortical layers, thus giving rise to the term ‘cortical columns’. The cortical columns are arranged in a continuous manner around singularities called pinwheels. On the other hand, neurons within the same horizontal plane (parallel to the cortical surface) tend to have increasingly different angle preferences as the distance between them increases. Although no such orientation maps were found in the rodent species studied so far, it has been proposed that the agouti’s visual cortex might be organized in this way (Weigand et al. 2017).

5.3.1 Changes of angle preference parallel to the cortical surface

In order to analyze the spatial clustering of neurons with similar orientation preference in agoutis (and compare it to the organization in cats) we analyzed the differences in neuronal orientation preference as a function of cortical distance. In cats, given the nature of orientation maps and the orientation columns that compose them, whenever comparing a pair of neurons, their orientation preference difference should increase smoothly with distance parallel to the cortical surface in all directions until targeting a proximal domain of orthogonal orientation preference. Similarly, the difference should be minimal when comparing adjacent cells, as within a cortical column. On the contrary, if the layout of the neurons in the ensemble would be random the similarity between neurons should be independent of the cortical distance.

We calculated the differences in orientation angle preference between neurons recorded with array electrodes (Figure 21). All the cortical distances shown are in fact distances between the electrodes at which the two neurons being compared were recorded. Therefore, the distances declared here are not exact neuronal distances, but an estimate of the distance between the electrodes at which the neurons were recorded. Whenever a comparison is declared to be of 0 μm distance, it means that the two neurons were recorded within the same electrode.
The first comparison measure we analyzed consists of the difference in the preferred angle of orientation. These angle differences range from 0 deg (for neurons with the exact same preferred stimulation angle) to 90 deg (for neurons tuned to orthogonal stimuli). Figure 21 shows the histograms of the angle differences separated for different discrete inter-electrode spacings for all single units recorded in cats and agoutis.

For cats, as expected, the histogram becomes more uniform as the comparison distance increases. For agoutis, only comparisons of neurons recorded within the same electrode (blue line) show a distribution different from the surrogate (calculated by shuffling the data). This suggests that even though agouti might not have a smooth orientation map, there is a higher similarity of neighboring neurons than what is predicted by chance.

Next, we wanted to visualize how this measure changes with cortical distance. Figure 22 shows the pairwise angle differences across horizontal (parallel to the cortical surface) cortical distance, for each of the SFs investigated separately. Although cat curves for different spatial frequencies show a larger dispersion than those of agouti.
(Figure 22) (most likely because we have fewer data points for cats) the general tendency is a smooth increase in the angle difference. We therefore analyzed them altogether for the statistic test (Figure 23).

Figure 22. Mean pairwise angle difference as a function of horizontal cortical distance for cat A18 and agouti V1 single neurons sampled from array electrodes (250 μm spacing). Data are plotted separately for different spatial frequencies (color code). Error bars are standard error of the mean (SEM). Data from 2 cats and 5 agoutis.

Figure 23. Mean pairwise angle difference as a function of horizontal cortical distance for cat A18 and agouti V1, taking into account the orientation preference at the highest OSI eliciting spatial frequency of stimulation. Asterisks depict Mann-Whitney p<0.05 for the pairwise comparison of the data points and the surrogate. Data from 2 cats and 5 agoutis.
<table>
<thead>
<tr>
<th>Distance (μm)</th>
<th>0</th>
<th>250</th>
<th>354</th>
<th>500</th>
<th>559</th>
<th>707</th>
<th>750</th>
<th>791</th>
<th>901</th>
<th>1061</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat A18</td>
<td>3.28e-30</td>
<td>1.94e-09</td>
<td>0.015</td>
<td>0.013</td>
<td>0.062</td>
<td>1.78e-04</td>
<td>7.42e-05</td>
<td>3.93e-04</td>
<td>0.381</td>
<td>1.29e-03</td>
</tr>
<tr>
<td>Agouti</td>
<td>9.17e-21</td>
<td>9.31e-03</td>
<td>2.03e-03</td>
<td>3.91e-03</td>
<td>0.44</td>
<td>0.34</td>
<td>0.33</td>
<td>0.06</td>
<td>0.67</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Table 3. Mann-Whitney p-values for pairwise comparisons of curve value versus the surrogate.

Figure 23 shows the angle difference as a function of horizontal cortical distance for all spatial frequencies analyzed considering, for each cell, the orientation preference obtained with the SF that elicited the highest OSI. The cat’s orientation preference difference curve shows the expected smooth increase across cortical distance, whereas the agouti’s curve is much flatter than that of the cat. From 250 μm on, angle differences remain around the estimate for a random distribution (dotted line). This suggests that agoutis lack a structure which is similar to cats. Nevertheless, most of the values were significantly different to the surrogate (Table 3). The most dissimilar value in the agouti curve is the one at 0 μm, which suggests that neurons recorded from the same electrode have a much lower angle difference, suggesting a local structure.

5.3.2 Changes of similarity index parallel to the cortical surface

Next, we calculated a response profile similarity index, which is the Pearson correlation between the firing rates to each stimulus orientation of the two neurons being compared. In contrast to the previous measure, this index takes into consideration not only the calculated preferred angle but also the shape of the each unit’s tuning curve (see methods). This is important because we observed that the orientation selectivity indexes of agoutis are much smaller than those of cats indicating that the calculated preferred angle might not be able to accurately reflect the overall functional layout.

For both cat and agouti data, response similarity decays with inter-electrode distance (Figure 24). However, for cats, the similarity index reaches values as high as 1 for the inter-electrode comparison and almost -0.5 for the largest inter-electrode distance...
(1061 μm) in the sample. This is to be expected as the distance between cross-orientation columns in cat area 17 is approximately 500 μm, and a little bit larger in area 18 (e.g. Löwel et al., 1987). In contrast, for agouti, the highest similarity index obtained reaches only 0.6 and negative values are never observed. Similarly to the previous analysis, we didn’t find clear qualitative differences between the SFs investigated (curves of different colors in Figure 24).

![Figure 24](image)

**Figure 24.** Similarity Index (Pairwise Pearson correlation of firing rates) for cat A18 and agouti V1 to stimulation angle as a function of horizontal cortical distance. Data from 2 cats and 5 agoutis.

Therefore, we analyzed all of the SFs together taking into account angle preference at the SF that elicited the highest OSI (Figure 25). In cats, this average similarity index decays rather smoothly and reaches negative values (Figure 25, blue curve). In agoutis, on the other hand, the coefficient’s decay over distance is much flatter and differs much less from its own surrogate data which represent the chance level (Figure 25, red curve). Whereas the cat’s similarity index differs significantly at all measured inter-electrode distances from chance level, the agouti’s differs only at three distances including the intra-electrode value.
Figure 25. Pairwise Pearson correlation of firing rates to stimulation angle as a function of horizontal cortical distance for cats’ A18 (A) and agoutis (B). Only best responses (highest OSI) are considered for each cell. Asterisks depict Mann-Whitney p<0.05 for the comparison of the data curve and the surrogate. Data from 2 cats and 5 agoutis.

<table>
<thead>
<tr>
<th>Distance (µm)</th>
<th>0</th>
<th>250</th>
<th>354</th>
<th>500</th>
<th>559</th>
<th>707</th>
<th>750</th>
<th>791</th>
<th>901</th>
<th>1061</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat A18</td>
<td>6.39 e-08</td>
<td>3.84 e-08</td>
<td>0.016</td>
<td>3.31 e-04</td>
<td>0.001</td>
<td>0.051</td>
<td>0.047</td>
<td>4.49 e-04</td>
<td>0.428</td>
<td>0.059</td>
</tr>
<tr>
<td>Agouti</td>
<td>1.32 e-11</td>
<td>0.239</td>
<td>0.011</td>
<td>0.247</td>
<td>0.977</td>
<td>0.798</td>
<td>8.92 e-04</td>
<td>0.289</td>
<td>0.059</td>
<td>0.304</td>
</tr>
</tbody>
</table>

Table 4. Mann-Whitney p-values for pairwise comparisons of curve value versus the surrogate.

From these plots, we draw the conclusion that the cat data demonstrate a spatial organization which is compatible with an orientation preference map, but that the agouti data are not. Only neurons that are situated close to each other (i.e. were recorded within the same electrode), have a higher similarity than chance, but this does not hold for neurons recorded at different recording sites. However, due to our recording technique (spatial arrays with a minimum inter-electrode distance of 250 µm) we could not sample at distances between 0 and 250 µm, and thus cannot determine the size of the microstructure that the high similarity index value suggests for neurons recorded within the same electrode. Noteworthy, the agouti’s similarity index never reaches negative values, also at larger distances, indicating that clearly segregated domains of orthogonal orientation preference are rather unlikely.
5.3.3 Angle differences in a cat intrinsic signal orientation preference map

One detail that worried us about the curves was the odd behavior of the data point corresponding to comparisons at 750 μm distance, for both distance measurements (Figures 22 and 24). In some occasions, this point seems to deviate abruptly from the expected trace connecting neighboring points. In an effort to understand whether this data point stems from a systemic artifact, we performed the same angle difference analysis with a cat orientation preference map obtained by intrinsic signal imaging (courtesy of Dr. Kerstin E. Schmidt). Applying the same analysis to this map should reveal an almost ideal distribution of angle differences of a cat’s orientation preference layout.

Figure 26. A: Cat intrinsic signal mapping including the transition zone between area 17 and 18. Angle preference is color coded according to the color bar on the right. Pixel distance = 25 μm [Courtesy of Dr. Kerstin E. Schmidt]. B: Mean and SD of angle difference comparisons, analogous to figures 22 and 23. ‘Pixel’ sampling refers to sampling at the mapping resolution. ‘Electrode’ sampling refers to sampling at distances equal to the array electrode geometry.

Figure 26A depicts said imaging map. Briefly, the angle preference of each pixel was obtained by analyzing the blood oxygen level-dependent signal change at different stimulus orientations. Subsequently, the preferred angle per pixel was computed by computing the vector sum of all different condition maps obtained by differently
oriented gratings. Figure 26B compares the angle differences computed for pairs of pixels (red line) and pairs of ‘electrode’ (black line). The ‘pixel’ sampling refers to comparisons at every distance possible, given all possible distances in a grid with 25 μm inter-pixel spacing (imaging resolution). The ‘electrode’ sampling refers to sampling only distances equal (or the closest possible) to those of the array device, but in the imaging map.

Both samplings match well, and start to deviate only at the farthest possible comparison, possibly because our ‘electrode’ sampling of the imaging is done with the borders of the putative device parallel to the borders of the image, therefore introducing a greater bias at larger distances. More interestingly, for ‘electrode’ sampling, the data point at 750 μm introduces a little break in the curve as observed with the real data sampled by the electrodes in the electrophysiological recordings. In contrast, the ‘pixel’ sampling (red line) does not show the break and descends smoothly. We hypothesize this is because of the portion of the curve this points are sampling. It coincides with the point where the ‘continuous’ curve starts decreasing, therefore it might be related to undersampling of a fast changing measure.

5.3.4 Comparison of response similarity parallel and orthogonal to the cortical surface

Inspired by the ‘mini-column’ findings in rodent visual cortex in the recent years (Ringach et al. 2016, Kondo et al. 2016, Maruoka et al. 2017), we investigated if the orientation preference similarity of nearby single units holds across the vertical dimension. To this end, we recorded with shank electrodes, and compared the units across either the shank dimension (Figure 27, blue, perpendicular to the cortical surface), or the array electrode dimension (Figure 27, red, parallel to the cortical surface). For both types of electrode and each spatial frequency analyzed, the inter-electrode angle difference (Figure 27, top) and similarity index (Figure 27, bottom) were calculated between all electrodes in the device where an OS unit response was recorded. Given the device geometries, for probe devices, comparisons included units as far as 1.6 mm apart in the vertical dimension (between the extreme recording sites of each shank). Similarly, for array devices, comparisons included units as far as 1.06 mm apart in the horizontal plane (between electrodes located at opposite vertices of each array).
For every statistical significant comparison, both for angle difference and similarity index, the units showed a greater similarity in orientation angle preference along the vertical dimension than in the horizontal plane. This is in accordance with the recent literature (Ringach et al 2016, Scholl et al 2016, Maruoka 2017) that describes clustering of similar orientation tuning in the visual cortex of rodents with interspersed organization on the micrometer scale. It also confirms that interspersed organization is not synonymous of random, and suggests that there is a vertical ‘columnar-like’ structure in rodent visual cortex.

Figure 27. Agouti orientation selective units similarity across vertical and horizontal dimensions. Pairwise comparisons across all electrodes of each device. Median and interquartile range are depicted by circles and lines respectively. Blue and red depict pairwise comparisons between sites from electrode arrays, or Michigan probes (shanks), respectively. Array electrodes sample from horizontal planes and shank electrodes sample from a vertical cylinder (relative to cortical surface). Top: angle difference metric. Bottom: Similarity index metric. Both metrics show a greater similarity between neurons arranged vertically. * depicts p<0.03 (Mann-Whitney U test). Shank electrode data from 3 agoutis. Array electrode data from 5 agoutis.
<table>
<thead>
<tr>
<th>Spatial Frequencies (cyc/deg)</th>
<th>0.04</th>
<th>0.08</th>
<th>0.16</th>
<th>0.32</th>
<th>0.64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angle Difference (SUA)</td>
<td>0.088</td>
<td>0.003</td>
<td>1.56 e-07</td>
<td>1.01 e-07</td>
<td>0.011</td>
</tr>
<tr>
<td>Similarity Index (SUA)</td>
<td>0.353</td>
<td>8.41 e-04</td>
<td>3.93 e-05</td>
<td>1.16 e-10</td>
<td>0.004</td>
</tr>
<tr>
<td>Angle Difference (MUA)</td>
<td>0.118</td>
<td>2.55 e-08</td>
<td>1.15 e-12</td>
<td>0.209</td>
<td>2.14 e-07</td>
</tr>
<tr>
<td>Similarity Index (MUA)</td>
<td>0.887</td>
<td>0.004</td>
<td>7.02 e-10</td>
<td>4.80 e-08</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table 5. Mann-Whitney p-values for pairwise comparisons of array versus shank electrodes. Bolded values correspond to asterisks depicted in Figure 27 (p < 0.03).
6. Discussion

In this study, we characterized visual responses and the spatial organization of orientation selective neurons in the agouti (*Dasyprocta agouti*). This is, to date, the largest and the only highly visual rodent studied besides the gray squirrel (Van Hooser et al. 2005). We used contrast reversing checkerboards and drifting sinusoidal gratings to visually stimulate the animals under anesthesia, while recording electrophysiologically in the primary visual cortex. In order to thoroughly compare our results, and validate our methods, we also performed the same experiments in cats (*Felis catus*).

6.1 Receptive fields

According to our results, agouti CRF sizes are in the 1-22 deg\(^2\) range. Previous agouti CRF mappings (Picanço-Diniz et al. 1991) report sizes of 8-33 deg\(^2\) close to the midline and of 30-71 deg\(^2\) in the temporal periphery, for units close to the horizontal meridian. The slight discrepancy with the previous literature might be explained because of our undersampling of the periphery, and also because of our different method to map the receptive fields. As opposed to our automatic method, Picanço-Diniz et al. performed all of their mappings by stimulation with hand-held cards. It is possible that the automatic mapping algorithm delineates the CRFs as smaller because of the 70% threshold.

The agouti CRFs also appear to be smaller than those reported for rats (7-130 deg\(^2\), Girman et al. 1999) and much smaller than those described for mice (50-700 deg\(^2\), Niell and Stryker, 2008). In contrast, agouti CRFs are in the low range of rabbit’s receptive field size (1-60 deg\(^2\), Murphy and Berman, 1979) which is more diurnal than rats and mice (Jilge, 1991).

6.2 Orientation and direction selectivity proportions

The orientation and direction selective cell proportions we found for cats in our experiments differ from the proportions reported in the literature. Cat proportions of OS and DS cells are reported to be 95-99% and 64% respectively (Table 1, Van Hooser...
2007). Although this is a considerable difference to our results (~75% and ~45% respectively), it is to be expected due to the variety of methods used along the decades and studies to define responsiveness, and orientation and direction selectivity. It is therefore more appropriate to, whenever possible, make a straightforward comparison within the same study, where all the data are acquired and analyzed in the same way, than to compare different studies in the literature.

Similar to the present work, Murphy and Berman (1979) performed a study of the visual cortex of rabbits and cats, where they report 99% of the recorded cells to be OS in the cat and 72% in the rabbit. Our methods show a similar difference in the proportion of orientation and direction selective cells for cat A18 and agoutis. Therefore, it appears that agoutis have less resources allocated to contour processing in the visual cortex than cats.

6.3 Orientation preference distributions bias

One of the main physiological differences between the visual systems of cats and agoutis is the different retinal organization. In cats (as in humans and plenty of other species), the location of highest visual acuity is a circular area termed area centralis. In contrast, agoutis have a visual streak: an elongated area that runs across the retina parallel to the visual horizon, which contains a higher density of ganglion cells, cones and therefore smaller receptive fields (Silveira et al 1989).

We found cats to exhibit a cardinal bias for orientation selectivity, while agoutis are biased towards horizontal contours. Although a horizontal bias has been described in the periphery of the visual field of primates (Yu and Rosa 2014), we hypothesize that the horizontal bias in the agouti is influenced by the retinal organization. The only exception is at the lowest spatial frequency tested, where vertical contours are preferred. The Agouti visual streak has a vertical width of ~10 deg around the horizontal meridian (Picanço-Diniz et al. 1991), which sets a limit to the width of horizontal stimuli they can accommodate in their area of highest visual acuity. We hypothesize this is why the bias in the orientation preference towards horizontal stimuli does not appear at SF=0.04 cyc/deg. At this SF, one cycle subtends 25 degrees of the visual field, which is far beyond what can be fitted within the visual streak.
This type of retinal organization allows for better vision in the visual field periphery and the bias towards horizontal contours implies larger resources allocated to detection of objects approaching or moving away from the animal. Similar to agoutis, rabbits have the same retinal organization and the same horizontal bias (Murphy and Berman, 1979). These morphological and functional specializations appear to correlate with the ecological niche occupied by both agoutis and rabbits as preys, because of the survival chances related to detecting predators approaching from a wide range of visual field eccentricities.

6.4 Direction preference distributions bias

Both cats and agoutis show a preference towards contours moving away from the vertical meridian and downwards in the visual field. This type of bias is usually called centrifugal, as opposed to the centripetal bias which involves preference to movement towards the vertical meridian or the fovea. This centrifugal bias has been described before in cats, especially for supragranular layers of the visual cortex, where most of our recordings were made (Bauer et al. 1989, Blakemore and Zumbroich 1987). In contrast, in rodents a centripetal bias has been described, such as for the California ground squirrel middle lateral and lateral visual areas, analogous to the PMLS area in cats and the MT area in primates (Paolini and Sereno 1998). A similar centripetal bias was also described for California gray squirrels (McCourt and Jacobs 1984) and gray squirrels (Blakeslee et al. 1985), in the direction selective ganglion cells in the optic nerve. The usual interpretation is that this bias is related to the recognition of objects moving towards the individual, such as predator detection. Conversely, a centrifugal bias, such as the one we found in agouti V1 is assumed to aid in the processing of locomotion. This interpretation of centrifugal bias is supported by the fact that agoutis are among the fastest running extant rodents.

6.5 Functional anatomy

Orientation preference maps (OPMs) are present in all primates and carnivores studied so far. Rodents and lagomorphs such as rats, squirrels and rabbits (Van Hooser 2007), on the other hand, show an interspersed organization of orientation selective neurons in their visual cortices. Although, this suggested a phylogenetic difference as the
reason for the observed differences, theoretical studies proposed the small V1 size as a reason for the lack of OPMs (Florez-Weidinger 2013, Weigand et al 2017).

Contrary to the theoretical predictions, our results in agouti, the largest rodent studied so far, are consistent with an interspersed organization. It is important to note, that interspersed does not equal random.

Strictly, we can only assert this claim for the monocular region of agouti V1 (where we recorded), but it is unlikely that the binocular region would show OPMs when the monocular region clearly does not. Although it has been proven that eccentricity (and therefore degree of binocularity) is relevant in determining spatial and temporal frequency neuronal tuning curves (in marmosets: Yu et al 2010); both monocular and binocular regions present OPMs in cats (Löwel et al 1987, 1988).

Recent studies have shown that a ‘mini-columnar’ structure of iso-oriented cells is present in mice (Ringach et al 2016, Kondo et al 2016). Our results are also consistent with a mini-columnar organization, being that neurons recorded in the dimension perpendicular to the cortical surface tend to be more similar in their orientation preference than neurons recorded in the dimension parallel to the cortical surface.

The mini-columns reported in mice have a radius of less than 50 μm, measured via 2-photon imaging at a high resolution. Our recordings from 1 megaohm electrodes sample from a radius of about 50 – 100 μm around the tip (Henze et al 2000), making it impossible to assert if the local structure we see in the agouti corresponds to the same scale as observed in mice. Further investigation of the agouti ‘mini-columns’ should focus on the range of the similarities, in order to understand if the iso-orientation domains are just as the ones observed in mice. If they were wider, the agouti could be in the frontier of a transition zone between animals with interspersed and OPM organization.

6.6 Concluding remarks

Our results show that agouti visual performance, in terms of spatial frequency bandwidth, receptive field size and orientation tuning width is on par both with that of animals that have orientation preference maps such as cats, ferrets and tree shrews
(Van Hooser 2007); and also with that of animals that do not exhibit OPMs such as gray squirrels and rabbits (Van Hooser et al 2005, Murphy and Berman 1979). Taken together, all of this suggests that OPMs do not substantially improve neuronal orientation tuning properties.

Much effort has been put in the past decades trying to find a visual attribute present in species with OPMs, and absent in species without OPMs. Yet, no such attribute has been found. As previously noted (Horton and Adams 2005), the inconsistency in the existence of orientation columns and OPMs across species provides circumstantial evidence in support of the hypothesis that columns (and OPMs) may be irrelevant for visual stimulus processing.

Nevertheless, the fact that processing might not be affected by the presence of maps does not mean that OPMs have no positive fitness. Wiring costs might play a substantial role in the development of neuronal functional architecture (Florez-Weidinger PhD thesis 2013). If this was the case, it would be expected that circuits that have a high homogeneity in their connectivity (iso-orientation connectivity) would present anatomical clusters of functionally similar cells.

In mice, orientation selective neurons receive inputs distributed over a broad range of preferred orientations (Jia et al. 2010). With such a wiring scheme, it would be equally (if not more) costly to arrange neurons in OPMs than in mini-columns or even randomly. As the connections become more selective, the clustering of iso-oriented cells would appear as a cost-effective solution for wiring length.

Therefore, OPMs might simply be an energy saving way of network organization that, as previous studies suggested (Florez-Weidinger PhD thesis 2013, Weigand et al 2017, Kaschube 2014), shows a transition between interspersed and orientation maps as connectivity schemes become more homogeneous.
7. References


Florez-Weidiger, 2013. Modeling the origins of spatial and temporal variability in visual cortical representations. PhD thesis with Prof. Dr. Fred Wolf at the Max Planck Institute for Dynamics and Self-Organization and Bernstein Center for Computational Neuroscience in Göttingen, Germany.


Zhao, C. et al., 2016. Differences in spatial and temporal frequency interactions between central and peripheral parts of the feline area 18. European Journal of Neuroscience, 44(8), pp.2635–2645.