**Introduction**

The cerebral cortex is a 6-layer sheet common to all mammals. Rather than molding a single structure/function relationship that is invariant across mammals, it is likely that natural selection has modified homologous cortical circuits such that they differ not only in structural details but also in operating principles. If this idea is correct, neuroscientists will have to uncover many families of structure/function relationships in order to understand the basic principles of cortical circuits.

One approach to discovering new structure/function relationships is to study a homologous cortical circuit in different species with unique features. Primary visual cortex has been identified in every examined mammal, from highly visual arboreal primates to the subterranean blind mole rat with subcutaneous eyes (Krubitzer and Kaas, 2005). In addition, the basic receptive field properties in mammalian visual cortex have been well studied: neurons in primary visual cortex of all examined mammals exhibit orientation selectivity, with varying amounts of direction selectivity, length summation or end-stopping, and invariance to stimulus position (simple vs. complex) (Van Hooser 2007).

Here, we have examined the progression of receptive field properties from the lateral geniculate nucleus of the thalamus to visual cortical layers 4 and 2/3 in the tree shrew *Tupaia glis*. We choose the tree shrew for 2 reasons. First, tree shrews exhibit an unusually exquisite cortical segregation of inputs from the LGN. Cortical layer 4 is divided into 2 tiers; layer 4a receives projections from ON cells in LGN layers 1 and 2, while layer 4b receives projections from OFF cells in LGN layers 4 and 5. LGN layers 3 and 6 exhibit ON-OFF responses, and project to the superficial layers 2/3. This segregation of cortical inputs is finer than any other species of which we are aware, and is likely to aid in understanding structure/function relationships in cortex.

The second reason we chose the tree shrew is that pilot data from another study (HR and DF) suggested that the tree shrew might lack simple cells, which are orientation-selective cells that respond to light or dark bars at specific positions, while having many complex cells, which are cells that respond to properly oriented bars of either sign at any location within their receptive fields. If true, this would represent a significant departure from receptive field properties observed in other mammals. In addition, this organization would violate the feed-forward model proposed by Hubel and Weisel. In their original paper describing simple and complex cells, Hubel and Weisel postulated that the position invariance of complex cells might be derived from the projections of many simple cells with different position preferences.

We found that the laminar organization of receptive field properties in layers 4 and 2/3 differs from that found in cat.

Properties don’t change much from LGN neurons

Orientation selectivity varies across layers, built up through the depth of cortex

Direction selectivity is nearly absent

**Methods**

Tree shrews ranging from 3 months to 1 year of age were anesthetized with a mixture of ketamine and xylazine (). A tube was inserted into the interperointal cavity for later delivery of neuromuscular blockers, a tracheostomy was performed, and the animal was inserted in a custom stereotaxic frame that did not block vision. All wound margins were infused with the long-lasting analgesic bupivicane (). Contact lenses (Platt Contact Lens) were inserted to protect the corneas. A small craniotomy (2-4 mm2) was made; in some experiments, the dura was left intact, while in other experiments, a small hole was made in the dura with a 31.5-gauge needle to ease the insertion of electrodes. At the conclusion of these procedures, tree shrews were paralyzed with the neuromuscular blocker pancuronium bromide () to suppress spontaneous eye movements, and ventilated with 0.5-2.5% isofluorane in a 1:1 mixture of nitrous oxide and oxygen. The animal’s EKG was continuously monitored to ensure adequate anesthesia, and the percentage of isofluorane was increased if the EKG indicated any distress.

We used fine-tipped carbon fiber electrodes (CarboStar-1 from Kation Scientific Inc) to record single units. In some initial experiments (N experiments, M units), we also used commercial tetrodes (Thomas Recording Inc) to record single units in layer 4 (Adams and Horton, 2006). We found that both electrode types could effectively isolate neurons in layer 4, although well-isolated neurons were encountered less frequently with carbon fibers than with tetrodes. Eventually, we used carbon fiber electrodes exclusively because they caused less dimpling in layer 2/3. Spikes on single channels or multiple channels were amplified with a preamplifier/amplifier system by Multichannel Systems () and acquired and clustered using a Micro1401 acquisition board and Spike2 software (Cambridge Electronic Design, LLC).

During each penetration, we identified the boundaries of the LGN or cortical layers as we advanced the electrode, and again as we retracted the electrode. To do this, we listened to the low frequency cortical “hash” while flashing the animal’s eyes with light from an opthalmascope, 0.5s on, 0.5s off. In the LGN, the layers progressed from 6 to 1 going from dorsal and lateral to ventral and medial (), and were identified by noting the dominant eye (6, 4, 3, and 1 are innervated by the contralateral eye, while 5 and 2 are innervated by the ipsilateral eye) and physiological responses (layers 5 and 4 exhibit OFF responses, layers 6 and 3 exhibit ON-OFF responses, and layers 2 and 1 exhibit ON responses) ().

The transition between cortical layers 2/3 and 4a was clear when we began to hear the dominant ON response in layer 4a, where axons from LGN layers 1 and 2 form synapses with cortical neurons. Similarly, the transition between layers 4a and 4b could be discerned when the hash response switched from ON to OFF, as LGN layers 4 and 5 project to layer 4b. Finally, we identified the boundary between layers 4b and 5 by the disappearance of the strong OFF hash responses, and continued advancing until we could identify the beginning of the white matter.

In some experiments, we made electrolytic lesions (5uA constant current for about 5 seconds, electrode negative) to verify our electrode positions within the LGN or cortical layers. Animals were perfused with 0.9% saline followed by 10% formalin, and brains were placed in 20% sucrose until they sank. Brains were then blocked, 50µm coronal sections were cut on a freezing microtome, and alternate sections were stained for Nissl or cytochrome oxidase so that lesions were easy to observe. In each case, the lesions were located in the layer or at the layer transition that was predicted by the cortical hash responses.

The depths of all cells and layer transitions were recorded digitally with a Sutter MP-285 manipulator. In some plots, cortical depths were combined across animals by projecting onto a “standard cortex” on a layer-by-layer basis. Depths of neurons recorded in layer 2/3 were normalized (linearly) to be between 0mm (surface) and 900mm (layer 4 border), neurons in layer 4 were normalized to be between 900mm and 1300mm, and neurons in layer 5 and below were normalized to be between 1300mm and 2200mm.

Visual stimuli were created in Matlab using the Psychophysics Toolbox () on a Macintosh G3 running OS9 and displayed on a Sony GDM-520 monitor (white point N, mean brightness M). Spike tuning curves were analyzed with custom software in Matlab.

**Results**

Our goal was to characterize how receptive field properties of neurons evolved across from the thalamus to the superficial layers of the cortex, so we sampled 1) the relay neurons in the LGN, 2) cells in cortical layer 4, and 3) cells in cortical layer 2/3. In order to make direct comparisons, we used the same protocol for all cells.

First, each cell’s receptive field location was determined by manually moving an oriented bar on the screen. Next, we coarsely sampled orientation selectivity with drifting square wave gratings (80% contrast, spatial frequency 0.2 cycles per degree (cpd), temporal frequency 4Hz, stimulus size 10° circular, 30° angle steps presented in pseudorandom order). We then measured responses to sinusoidal gratings at different spatial frequencies (orientation set to the preferred orientation) and temporal frequencies (orientation and spatial frequency set to preferred values). Subsequently, we made a “fine” measurement of orientation and/or direction selectivity by sampling responses to drifting sinusoidal gratings (22.5° angle steps, spatial and temporal frequencies set to preferred values). In some cells, we also examined contrast responses with drifting gratings.

Once we had established the cell’s orientation selectivity with fine resolution, we measured the contribution of ON and OFF inputs by measuring responses to thin bars. The bars were as long as the screen permitted (greater than 20° in length) and narrow (0.25-0.5° wide). The center location of the bar was varied in 0.25-0.5° steps orthogonal to the preferred orientation, and the color was varied, either white (to measure ON responses) or black (to measure OFF responses) on a gray background. Oriented bars were used here instead of black or white spots because the vast majority of cells in our study responded reliably to bars, whereas many cortical cells did not exhibit robust responses to small spots.

*ON/OFF responses, modulation, and orientation selectivity in LGN*

As expected, neurons in layers 1, 2 and 4, 5 in the lateral geniculate nucleus exhibited strong ON or OFF responses in the center of their receptive fields, as shown in Figure 1a. Also, as expected from previous work (Kuffler), these ON- or OFF-center cells gave spiking responses to the opposite polarity at the receptive field edges (corresponding to the surround of the LGN receptive fields). Similar to LGN cells in other mammals, tree shrew LGN neurons were highly modulated by drifting sinusoidal gratings, and tended to respond with sinusoidal output (Figure 1b). Finally, these LGN cells tended to exhibit very low orientation selectivity, that is, they responded equally well to gratings with different orientations (Figure 1c).

To quantitatively compare these responses with those of cortical neurons, we developed several index values (Figure 1, Figure 2). We created a normalized ON/OFF index that varied between 0 (ON) and 1 (OFF), computed as ON/OFF index = OFF response / (ON response + OFF response). In addition, we made a Sign index that indicated to what degree the cell was balanced in ON or OFF responses (near 0), or favored a particular sign (near 1): Sign index = | ON response –OFF response | / (ON response +OFF response).

For sinusoidal grating responses, we calculated a modulation index, 2 \* F1 / (F1+F0), where F1 is the magnitude of the Fourier coefficient of the response at the stimulus temporal frequency (that is, the magnitude of the sinusoidal component or the modulated component), and F0 is the mean response (DC response, or the unmodulated component). This modulation index can vary from 0 to 2, and is 1 when the F1 and F0 components are equal. In previous studies, cells have been classified as “simple” when the F1 component of an orientation-selective cell is equal to or larger than the F0 component, and as “complex” if the F0 component is larger than the F1 component.

Finally, we defined an index of orientation selectivity. Many cells in the lateral geniculate nucleus did not exhibit much orientation selectivity, so we wanted to choose an index that did not depend on Gaussian fits or the orthogonal to preferred ratio, as these quantities could indicate spurious peaks (that is, peaks due to noise) in cells with low orientation selectivity. Instead, we chose a vector-based index, 1 minus the circular variance (Ringach et al., 2002), or (1-CV = |R|) as an index of orientation selectivity that was robust for cells with either low or high orientation selectivity. This value can vary from 0, if a cell responds equally at all orientations, to 1, if a cell responds for a single orientation only.

*ON/OFF responses, modulation, and orientation selectivity in cortex*

Single neuron responses in cortical layers 4a and 4b were remarkably similar to responses in LGN layers 1, 2 and 4, 5, respectively. Layer 4a cells tended to exhibit strong ON responses, while neurons in layer 4b exhibited strong OFF responses (Figure 1b). Further, cells in layers 4a and 4b were highly modulated by drifting gratings. As shown in Figure 2, there were no significant differences between Sign Index values or Modulation Index values between cortical layers 4ab and LGN layers 1, 2, 4, and 5.

The only major difference between LGN cells and cells in cortical layers 4a and 4b was that cells in layers 4a and 4b exhibited modestly stronger orientation selectivity (Figure 1c, 2c, p=NNN). The median orientation selectivity value was NNN in layers 4a and 4b, while it was only MMM in LGN 1, 2, 4, and 5.

Neurons in layers 2/3 often exhibited strong responses to both ON and OFF stimulation at the same receptive field location (Figure 1a). Rather than exhibiting modulated responses, cells in layers 2/3 most commonly exhibited more constant (unmodulated) responses (Figure 1b), and had a median modulation index value of NNN. Finally, orientation selectivity was dramatically increased in layer 2/3 as compared to layers 4a and 4b; the median orientation selectivity value was XXX (Figure 1c).

The cumulative distributions in Figure 2 do not allow one to observe any finer-scale changes within the cortical layers, or to appreciate the diversity of the cortical responses within the layers. The index values for all cells in our study and their normalized depth within cortex (see “Methods”) or layer within the LGN are shown in Figure 3. There were 3 particularly notable features in this data.

First, one should consider that some of the variation in these responses may be due to the resolution with which we are able to identify each cell’s depth within cortex. For example, we are not likely to be able to localize cortical layer boundaries with a accuracy greater than 50µm. In addition, it is possible that, for some cells, we recorded spikes from dendritic or axonal segments rather than the soma, and the soma may be located hundreds of microns away from our recording site. Nevertheless, we were able to observe the known sublaminar organization of cortical layer 4, as strong ON biases are found in layer 4a, which abruptly change to strong OFF biases in layer 4b. This result gives us confidence that our cell localization procedures are accurate enough for us to draw conclusions at the resolution of the layer 4 sublayers.

Second, while a vast majority of (but not all) neurons in LGN and layer 4 of cortex were highly modulated by drifting sinudoidal stimuli, the situation was more complicated in layer 2/3. The median cell did not exhibit strong modulation, but there was a substantial minority of cells that were highly modulated, and the modulation index across the population was more variable than in LGN or cortical layer 4.

Third, we observed an unexpected pattern in the progression of orientation selectivity through the cortical layers. While LGN cells exhibited little orientation selectivity, the situation in cortical layer 4 was more complicated. The median orientation selectivity in layer 4 was slightly higher than that found in the LGN, but a few cells in layer 4 exhibited strong orientation selectivity. In addition, there was a region of increased orientation selectivity at the top of layer 4a. Finally, it was not surprising that orientation selectivity in layer 2/3 was much stronger generally than in layer 4 (Chisum et al., 2003), but we were surprised to observe that orientation selectivity dramatically increased in strength from deep layer 2/3 to superficial layer 2/3.

*Simple and complex cells in tree shrew V1*

Orientation-selective cells have traditionally been divided into 2 classes: simple cells, which respond to light and/or dark bars at separate and specific spatial positions, and complex cells, which respond to either light or dark bars at any position within the cell’s receptive field. Simple cells are highly modulated by drifting sinusoidal gratings, owing to their separated ON/OFF receptive fields, while complex cells typically exhibit a constant (unmodulated) response to drifting gratings.

For some years there has been uncertainty as to whether or not tree shrews might have simple cells. Previous characterizations of layer 4 indicated that layer 4 cells exhibited much less orientation selectivity than layer 2/3, and non-oriented cells are not simple cells in the classical sense. Further, the majority of cells that have been recorded in layer 2/3 have exhibited complex receptive fields. This led to speculation that tree shrew receptive fields may go from being unoriented in layer 4 to being oriented and complex in layer 2/3. Such an abrupt transition would violate the classic feed forward model of Hubel and Wiesel, who postulated that the position-invariance of complex receptive fields are built up via input from many simple cells that each respond at different positions.

Orientation selectivity and modulation indices for the neurons in our population are plotted in Figure 4. As in other mammals, a majority of LGN cells exhibited high modulation index values and low orientation selectivity, and only 2 of M neurons showed an orientation selectivity greater than 0.2 and a modulation index value greater than 1. However, 8 of 14 layer 4 cells could be classified as simple by this criteria. Further, layer 2/3 responses were more variable; 13 of 44 cells exhibited orientation selectivity and modulation index values that were considered simple, while a small majority of cells (23 of 44) were orientation selective and unmodulated, that is, classically complex.

*Spatial frequency, temporal frequency and contrast responses*

Another hallmark of the transformation of receptive fields from the LGN to cortex is an increase of the percentage of neurons that exhibit “bandpass” spatial frequency tuning; that is, these cells do not respond to drifting gratings with low spatial frequencies, and instead are selective for intermediate frequencies and then have a high-frequency cut-off.

Spatial bandwidth – matches other animals?

Temporal frequency tuning was very similar to that of other diurnal mammals, which, as a group, exhibit higher temporal frequency preferences than nocturnal animals (Heimel et al., 2005). Temporal frequency properties did not change much from LGN to cortex.

Contrast gain values in some species exhibit higher non-linearities in cortex than in LGN.

**Discussion**

Tree shrew cortex appears to be organized in a similar manner to other mammals, albeit with a stronger segregation of receptive field properties within the cortical layers.

So simple cell receptive fields have now been described in every animal where they have been sought, including rodents, carnivores, primates, marsupials, and now tree shrews (order Scandentia).

Nevertheless, the results of this study also indicate that the way orientation selectivity is built up surely varies across mammals.

**References**

Blah