**Orientation biases in the Tree Shrew Superior Colliculus neurons.**

**Summary**

Though earlier theories of orientation selectivity suggested that orientation biases observed in V1 inputs are the result of excitatory convergence, studies have shown that these biases may be inherited from neurons in sub-cortical structures, namely the lateral geniculate nucleus (dLGN) and ultimately the retina. However, there is some controversy as to whether the biases reported in these sub-cortical structures arise from cortical feedback instead of biased inputs. If orientation selectivity arises from the retina, it should be evident in other targets of retinal projections. The superior colliculus (SC) is one such area. Here, orientation selectivity of SC neurons in tree shrews was measured using thin bars and gratings of different spatial frequencies. SC neurons showed orientation tuning and spatial frequency tuning comparable to that observed in the LGN of tree shrews. At higher spatial frequencies, the orientation selectivity was more evident, similar to that reported in the retina and LGN of cats and macaques. Similar results have also been reported in layer 4 of the tree shrews earlier in this thesis (see Chapter 5). These results indicate that the potential source of orientation biases in the LGN and the SC could be the retina. Direction selectivity and linearity of the SC neurons were also studied.

**Introduction**

The tree shrew superior colliculus (SC) is a large well laminated structure (Tigges & Shanta, 1969; Zhou et al., 2016). It is sub-divided into areas important for visual form processing and visuomotor processing (Casagrande et al., 1972; Casagrande & Diamond, 1974) and has been implicated in an alternative pathway to the visual cortices (Killackey et al., 1971; Killackey & Diamond., 1971). While the functional role and the projections of the SC have been extensively studied, few studies have characterised the receptive field properties of individual neurons. In this chapter, the receptive field properties, specifically orientation and spatial frequency tuning of the tree shrew SC neurons were studied and compared with the properties of the geniculo-striate system of the tree shrews.

Due to its extensive reciprocal projections to sensory as well as motor areas of the brain, the SC was implicated in oculomotor behaviour (Sherrington, 1947). However, studies where different layers of the SC were lesioned showed that the SC consisted of two separate functional systems --- the superficial layers essential for visual form discrimination and the inferior layers implicated in oculomotor function and orienting behaviour (Casagrande et al., 1972; Casagrande & Diamond, 1974). The superficial layers of the tree shrew SC are further divided into stratum zonale (SZ), stratum griseum superficiale (SGS) and stratum opticum (SO) with the SGS further subdivided into upper and lower SGS (uSGS and lSGS respectively). As these are the layers of the SC that predominantly receive visual input (from the retina and primary visual cortex (V1); see May, 2006 for review), only the response properties of neurons in these superficial layers were studied.

In the present study, we aimed to examine orientation biases in the tree shrew SC. More than 50 years after its first report, the mechanism underlying orientation selectivity is still debated (Ferster & Miller, 2000; Priebe & Ferster, 2008; Scholl et al., 2013; Vidyasagar & Eysel, 2015; Kremkow et al., 2016). The model of excitatory convergence proposed by Hubel & Wiesel (1962, 1968) for orientation selectivity in cats and macaques assumed that orientation selectivity was first generated in the primary visual cortex along the visual pathway. A similar mechanism, where orientation selectivity is generated for the first time in layer 2/3 of the primary visual cortex has been proposed in the tree shrews (Chisum et al., 2003; Mooser et al., 2004) These theories have largely ignored the orientation biases that have been demonstrated in sub-cortical areas. Subcortical orientation biases have been reported in the retina and the dLGN of most species (cats- Levick & Thibos, 1980; Vidyasagar & Urbas, 1982; Levick & Thibos, 1982; Shou & Leventhal, 1989; macaques- Smith et al., 1991; Xu et al., 2002, Passaglia et al., 2002; tree shrews- Van Hooser et al., 2013; rodent- Tan et al., 2011; Sun et al., 2016). However, oriented neurons in the superior colliculus have only been reported in rodents (Wang et al., 2010; Inayat et al., 2015; Ahmadlou et al., 2015; Shi et al., 2017). In cats and macaques, direction selectivity has been reported in the Superior Colliculus (McIlwain & Buser, 1967; Sterling & Wickelgren, 1969; Rosenquist & Palmer, 1971; Cynader & Berman, 1972; Goldberg & Wurtz, 1972), but units can be selective to direction without being selective to orientation (see figure 6a and 6b in results). In one detailed study that examined receptive field properties in the tree shrew SC, a small proportion (~20%) of SC neurons were orientation tuned (responded three times better at optimum orientation compared to the non-optimum orientation) in the superficial layers of the SC (Albano et al., 1978). A recent study in the tree shrew geniculostriate system however, showed that nearly 50% of tree shrew LGN neurons showed orientation biases (Van Hooser et al., 2013), albeit to a smaller extent than that reported by Albano et al. (1978). In this chapter, the orientation biases of the shrew SC were characterized using bars and gratings and compared to that of the shrew LGN.

Since neurons in the superficial SC receive inputs from both retina and the primary visual cortex, we further characterized the receptive field properties of the SC neurons so as to infer their source. Certain properties of the SC neurons such as binocularity, direction selectivity and colour selectivity have been attributed to cortical feedback in carnivores and primates (Sterling & Wickelgren, 1969; Cynader & Berman, 1971; Tailby et al, 2012). In the rodents however, it has been shown that direction and orientation selectivity were inherited directly from the retinal projections on to the SC neurons (Shi et al., 2017). Therefore, one key aim of this experiment was to determine whether the receptive field properties of SC neurons were more similar to those of the retinal neurons or cortical neurons. Neurons of the primary visual cortex show sharp orientation selectivity and a bandpass spatial frequency tuning (Movshon et al., 1978a; Movshon et al., 1978b; Movshon et al., 1978c; DeValois et al., 1982). Retinal and LGN neurons are broadly tuned to orientation and have a low pass spatial frequency tuning. At higher spatial frequencies, neurons from both regions show sharper orientation tuning (Enroth-Cugell & Robson, 1966; Levick & Thibos, 1980; Levick & Thibos, 1982; Vidyasagar & Urbas, 1982; Vidyasagar & Heide, 1984; Shou & Leventhal, 1989). Here, we examined the orientation and spatial frequency responses of the neurons of the superior colliculus.

As we propose that asymmetries in the feedforward signal are elaborated by the target neurons to elaborate feature selectivity (Vidyasagar & Eysel, 2015), we predicted that the SC and the LGN neurons in the tree shrews, will respond similarly. Accordingly, the following hypotheses were tested.

**(H1)** Superficial SC neurons will show oriented responses when shown thin bars (which contain high spatial frequency information).

**(H2)** Superficial SC neurons will have low pass spatial frequency tuning, similar to their LGN counterparts, when tested using sinusoidal gratings.

**(H3)** When gratings of different spatial frequencies are used, a similar proportion of superficial SC and LGN neurons will be orientation tuned. The SC neurons will also show an orientation selective response at higher spatial frequencies.

**Methods**

**Surgery and anaesthesia**

Surgical procedures have been outlined in detail in the Methods chapter. Briefly, the animals were anaesthetized using a mixture of Ketamine and Xylazine, a venous catheter was inserted in to the femoral vein and a tracheostomy performed to assist in breathing during the experiment. The animal was administered muscle paralysant (Vecuronium Bromide) intravenously and was anaesthetised using Isoflurane (0.5-1%) for the duration of the experiment. Hard contact lenses were fitted to the eye to prevent corneal drying. A craniotomy and durotomy were performed over the location of the SC (Horsley-Clarke Co-ordinates A2.5 to P2.5). Frontal EEG and ECG were monitored during the experiment. At the end of the experiment, the animal was euthanized using an overdose of pentobarbital sodium and perfused (using 0.1M Phosphate Buffer (PB) solution followed by 4% Paraformaldehyde in 0.1M PB), the brain was removed and stored in sucrose (20-25%) for histology.

**Electrophysiology**

High impedence, lacquer coated tungsten microelectrodes (FHC Metal Microelectrodes Inc., ME, USA; impedance= 12-18 MΩ) were lowered into the brain and the signal was amplified and filtered (x 10,000 gain, bandpass filtered between 300-3000 Hz, A-M systems) and fed into an audio speaker as well as an analog to digital converter (Cambridge Electronic Design Limited, Cambridge, UK; digitised at 22.5 kHz). The SC was identified by listening to the neuronal activity in the speaker. The electrode was first quickly descended to a depth of 3 mm and then slowly descended until visual neurons were identified. Lesions (6 μA for 6s) were made at the end of each track. When thehe electrode was withdrawn and lesions were made at regular intervals to trace the path of the electrode through the brain. The data was recorded using the spike 2 software (CED, Cambridge, UK). The spikes were templated and the spike timing exported as a text file. Further analysis was performed using custom MATLAB code (The Mathworks Inc, USA).

**Stimuli**

Stimuli were presented using a BARCO monitor (Frame Refresh Rate= 80 Hz; Reference Calibrator Plus; Barco Video and Communications, Belgium) centred on the neuron’s receptive field and generated using Visage (VSG, Cambridge Research Systems, Cambridge, UK) and custom Stimulus Description Language (SDL) scripts. The monitor had a mean luminance of 32.6 cdm-2. While recording, the monitor was placed at a distance of 114 cm from the eye. For each of the different stimuli described below, ten complete stimulus presentations were completed.

For each SC neuron, the preferred stimulus orientation was initially measured using a thin moving bar. The bar was presented in 9 different orientations sweeping bi-directionally (a total of 18 orientations). The background was a uniform gray screen. Depending on the polarity of the neurons, either a bright bar or a dark bar was used (contrast= 100 %). The bar was usually 8o long (ranging between 4 and 8 degrees) and 0.5o wide (ranging between 0.1 and 1 degree). The velocity of the bar was between 5 and 20 o/second. The width of the bars were usually reduced until an oriented response was observed. Where the thinnest bar we presented did not elicit an oriented response during the experiment, the neuron was classified as unoriented.

Peri-stimulus-time-histograms (averaged over 10 trials, 20 ms bin-width; PSTHs) were generated using the spike 2 software for online analysis. Based on the PSTHs generated following the presentation of the bar, the optimum orientation of the neuron was determined and used for further testing.

The spatial frequency responses to gratings were then measured. The animals were presented with drifting sine-wave gratings (Temporal Frequency= 4Hz; Contrast=100%) of varying spatial frequencies (SF; SF between 0 cycles per degree (cpd) to 2 cpd) at atleast two different orientations (optimum, optimum + 90o). Where we could perform stable recordings, SF responses at two more orientations (optimum+45 o, optimum-45 o).

**Data Analysis**

Regardless of the stimulus presented, the following analysis was performed on the extracellular trace before any specific analysis was undertaken. Spikes were templated and the spike time and stimulus markers were exported into text files. Using custom scripts in MATLAB, PSTHs (bin-width= 20ms) were constructed for each of the stimulus conditions. Spike density functions (SDFs) were created using a moving Gaussian envelope with σ of 60 ms (3 bins). This SDF was used for further analysis.

Analysis of Bar Stimuli

For orientation tuning recorded using a bar, the peak response in the SDF for each direction of movement was plotted on a polar diagram. The circular variance (CV; Ringach et al., 2002) and the orientation bias (bias, Vidyasagar & Urbas, 1982) were also calculated as follows:

CV= ………………………………………………………………(1)

Where θis the direction of movement of the bar (between 0 and 340 degrees) and r is the response at that direction. A CV value of 0 meant that the neuron was sharply tuned to orientation and a circular variance value of 1 meant that the neuron responded equally at all orientations. In this study, neurons with a CV greater than 0.9 were classified as unoriented neurons (Ringach et al., 2002).

Bias= ……………………………………………………………………………..(2)

Where Ropt is the response of the neuron to the optimum direction of movement and Rorth is the response of the neuron to the orientation 90 degrees away from the optimum direction of movement. Unoriented neurons have a value close to 1 and oriented neurons can have bias values upto Infinity.

Direction selectivity of the neurons was also calculated using two different methods to enable comparison with previous studies in the superior colliculus. First, the direction selectivity index (DSI) was calculated by taking the ratio of the response at the optimum direction of movement and the response at the opposite direction of movement (Goldberg & Wurtz, 1972). Neurons whose DSI was less than 0.5 (i.e. response in the opposite direction less than half of the response in the optimum orientation) were classified as direction selective. In the second method, the following formula was used to measure the directional circular variance (DCV; VanHooser et al., 2013).

DCV= ………………………………………………………….....(3)

Conventions are as described for the calculation of circular variance (Equation 1). Once again neurons that had a DCV less than 0.5 were not direction selective.

Analysis of Grating Stimuli

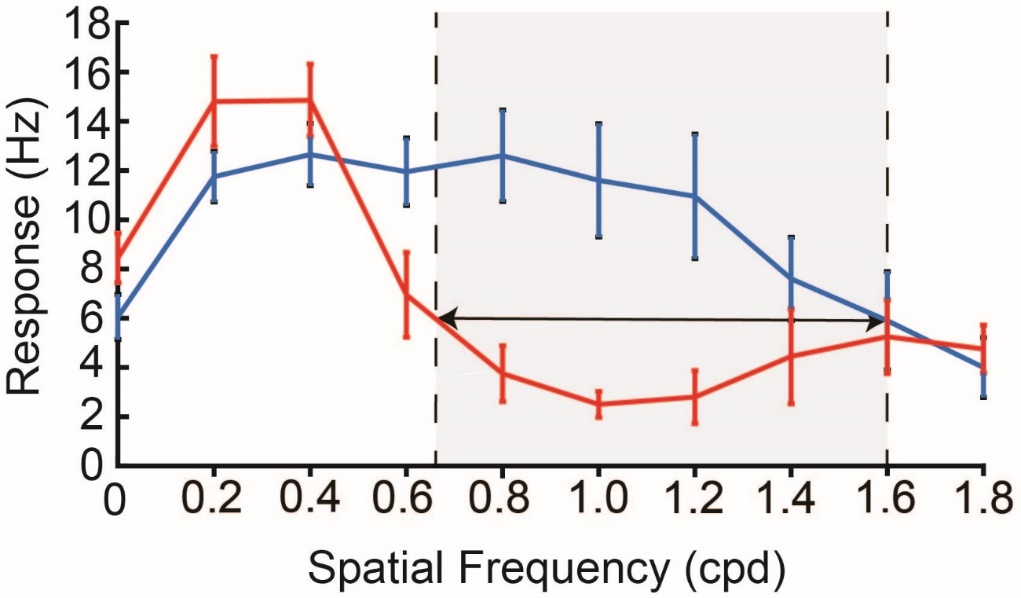
For gratings, the Discrete Fourier Transform (DFT) of the spike density function was calculated using the MATLAB Fast Fourier Transform algorithm (FFT). The F1 and the F0 components of the response were calculated (see General Methods for details) and the modulation index (Van Hooser et al., 2013) was calculated as follows:

Modulation ratio= ……………………………………………………………..(4)

Where F1 is the value of the modulated component at the peak spatial frequency and F0 is the value of the unmodulated component at the peak spatial frequency. If the modulation ratio was less than 1, the cell was considered to show non-linear summation over its receptive field and the unmodulated component of the response was used for further analysis. If the ratio was greater than 1, the cell was considered to show linear summation and the F0 component of the response was used.

In order to characterize the spatial frequency tuning response of the neurons, the peak spatial frequency of the neuron was taken as the spatial frequency where the firing rate was maximum. In most cases, the F0 and F1 components of the response peaked at the same spatial frequency. However, in some cases, the peak spatial frequencies of the F1 and F0 components were different. In these cases, if the F1 response was significantly greater than the F0 response, the peak spatial frequency of the F1 response was used. Otherwise, the peak spatial frequency of the F0 response was used. The lower cut-off was the frequency lower than the peak spatial frequency that gave a response that was half the magnitude of the peak response. If the response did not reach half the maximum response, the neuron was classified as a low pass tuned neuron. The high cut-off was the spatial frequency higher than the peak spatial frequency where response was half the magnitude of the peak response. The spatial frequency tuning bandwidth was then the difference between the high cut-off and the low cut-off spatial frequencies.

In order to see if the neurons showed sharper orientation tuning at higher spatial frequencies, first the spatial frequency tuning curve at the optimum and orthogonal orientations were generated and the bandwidth where the superior colliculus neurons responded for the optimum orientation but not for the orthogonal orientation was calculated. In order to do this, a ‘minimum response’ was defined as the response where the neurons fired similarly for the optimum and the orthogonal orientations. The spatial frequencies where the response rate for the optimum and orthogonal orientations first reached the minimum response were termed the optimum cut-off and orthogonal cut-off and their difference was calculated (see figure 1).



*Figure 1:* *Example SF tuning curves for optimum (blue) and orthogonal (red) orientations. The cut-off frequency at the optimal orientation is the SF at which the response at optimal orientation is no longer significantly different from the response at orthogonal orientation. The response at the cut-off frequency for optimum orientation is called the minimum response. For the orthogonal orientation, the cut-off frequency was the SF at which minimum response was first reached. Error bars are 95% confidence intervals.*

Circular variance of the neurons at each spatial frequency was also calculated using Equation 1 where spatial frequency tuning data for at least four different orientations was recorded. The orientation selectivity index (OSI) at each spatial frequency was also calculated as 1- reciprocal of orientation bias (Equation 2). The OSI instead of the bias was calculated in this instance as 1) no comparison to previous studies were made using this data; 2) The value of OSI would always be within 0 and 1 whereas the maximum value of bias could be infinity; and 3) As this calculation only requires the spatial frequency data at the optimum orientation and orthogonal orientations, a more complete data set to examine the relationship between orientation tuning and the spatial frequency tuning was present. The relationship between the OSI and CV were also examined in the results.

**Histology**

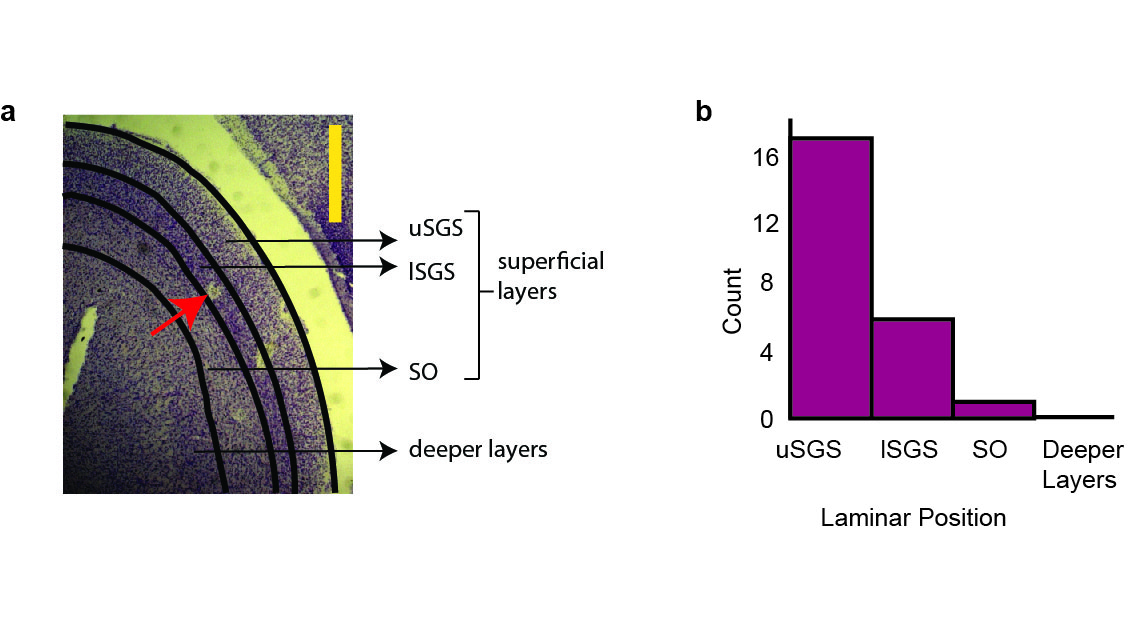
The brain that was stored in the sucrose at the end of the experiment, was cut into 50 micron sections using a cryostat and then mounted on gelatinised slides. The sections were then stained using Cresyl Violet acetate solution. Lesions were identified and the electrode tracks reconstructed using Adobe Illustrator to verify that all our neurons were indeed recorded from the superior colliculus.

**Results**

A total of 22 units were recorded from five tracks in three anaesthetised Tree Shrews (2 female and 1 male). All neurons were from the superficial layers of the SC. Of the 22 neurons, 20 were biased for orientation. Spatial frequency tuning information was collected for 16 units, 12 of which showed low pass spatial frequency tuning. 13 of the 16 neurons also showed sharper orientation tuning at higher spatial frequencies.

**Anatomical location of units**

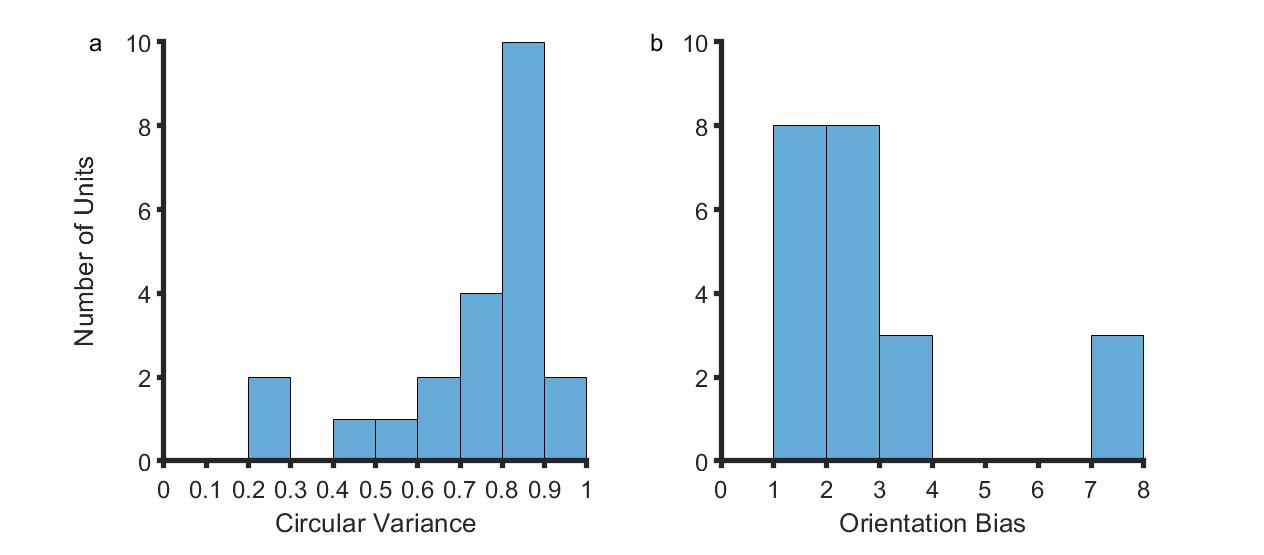
The laminar position of all the units was determined by reconstructing the electrode tracks from the electrolytic lesions. The photomicrograph of a Nissl stained section from the SC is presented in figure 2a. The laminar position of the neurons determined from the electrode track reconstructions is shown in Figure 2b. All the recorded neurons were from the superficial layers, with the majority from the SGS.



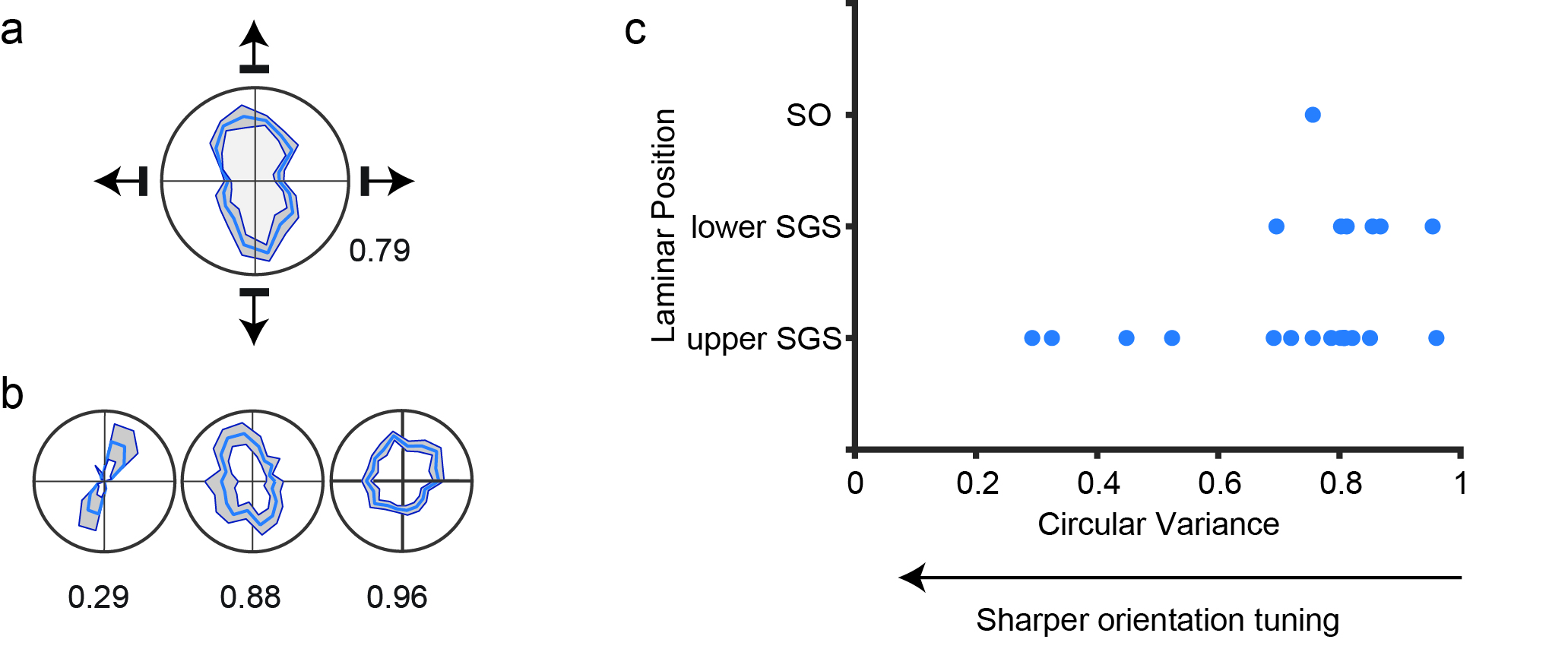
*Figure 2:* *Laminar position of the neurons. (a) A photomicrograph of a tree shrew superior colliculus from the right hemisphere showing the different subdivisions of the superficial layers. The red arrow points to a lesion. Two other lesions from a different track are visible to the right of the lesion. The yellow scale bar is 1mm. (b) Number of cells sampled from each layer. Majority of the cells were from uSGS. Abbreviations: uSGS- upper Stratum Griseum Superficiale; lSGS- lower Stratum Griseum Superficiale; SO- Stratum Opticum.*

**Orientation Selectivity using bars**

The distribution of two measures of orientation selectivity are shown in Figure 3. Figure 3a shows the distribution of circular variances. The median circular variance for the sample was 0.80 (95% confidence interval(CI)= [0.70, 0.82]). The orientation tuning curves of a representative neuron as well as those of the most selective, least selective neuron with CV less than 0.9 and the least selective neuron in the entire sample are presented in figure 4a and b.



*Figure 3*: *Orientation selectivity of neurons (a) This figure shows the distribution of circular variances of all neurons. (b) This figure shows the distribution of orientation biases.*



*Figure 4: Orientation selectivity when measured with bars. (a) The orientation tuning of a representative neuron. The direction of movement of the bars are shown at the poles of the plot. The number next to the plot is the circular variance. The maximum firing rate of the neuron was 117 spks/s. (b) Orientation selectivity of the sharpest, least tuned neuron included for further analysis and the least tuned neuron in the entire sample. The direction of movement of the bar are similar to (a). The circular variance of each neuron is displayed below. Maximum firing rate for the three neurons are 18 spks/s, 57 spks/s and 89 spks/s respectively. The response shown is the average of 10 trials and the error bars are ± 2\*SEM. (c) the distribution of circular variances of all neurons in relation to the laminar position of the neuron.*

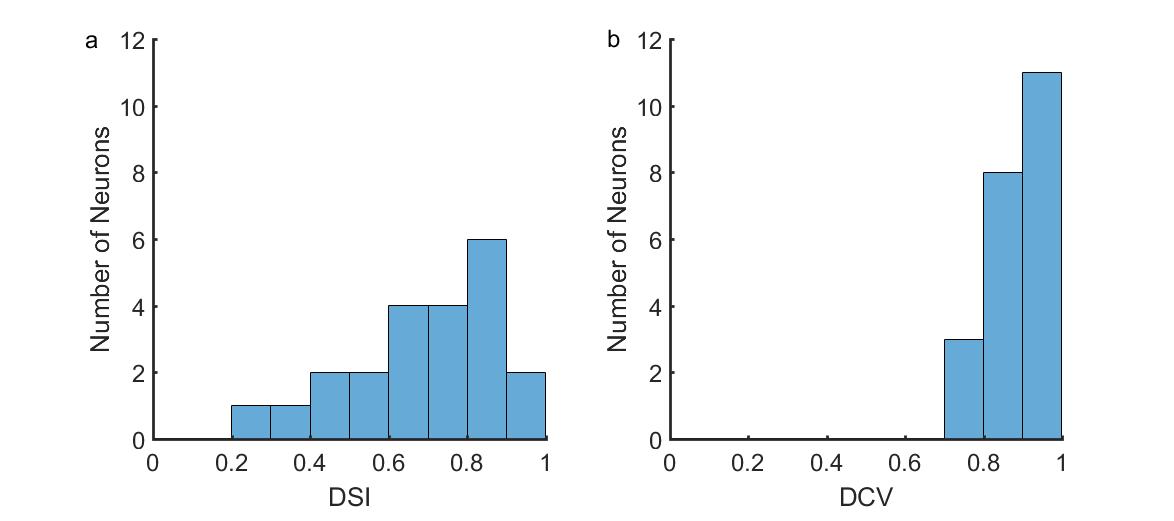
Any neuron with CV greater than 0.9 was classified as not selective to orientation. Two neurons had a CV greater than 0.9 and hence were not further recorded from.

An additional measure of orientation selectivity, the orientation bias (Bias; figure 3b) was also calculated. The median bias was 2.31 (95% CI=[1.85, 3.20]). A bias of one would indicate that the response of the neuron at the optimum and orthogonal orientations were the same. Therefore, lower values of bias indicated that the neurons were more broadly tuned. The two neurons that had circular variances greater than 0.9 had bias values closer to 1 (1.16 and 1.26). The orientation bias was calculated to enable comparison with previous studies and further analysis was not conducted using these values.

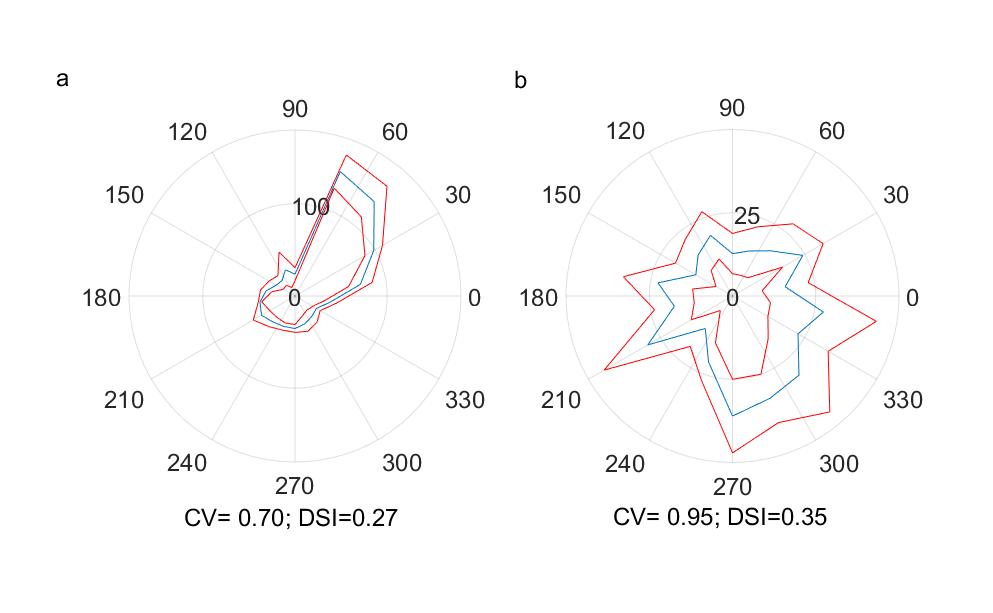
We also examined if there were any laminar differences in the orientation biases, the circular variance of the neurons were also plotted against the laminar position in figure 4c. The neurons that showed the sharpest orientation tuning were all located in the uSGS.

**Direction selectivity of neurons**

The distribution of the DSI and the DCV of 22 neurons are shown in Figure 5a and b. All 22 neurons were included in the analysis as neurons that are not tuned to orientation can be tuned to direction. Figure 6a shows a neuron that is selective to both orientation and direction. 6b shows a neuron selective to direction but not to orientation. The median DSI was 0.69 (95% CI= [0.5, 0.83]), suggesting that the majority of the neurons were not direction selective. Of the 22 neurons that were recorded from, only 5 (~20%) satisfied our criteria for direction selective neurons. The distribution of the DCV is shown in figure 5b. The median DCV was 0.90 (95% CI= [0.85, 0.93]). The DCV was a more conservative measure of direction selectivity and none of the neurons we measured from were selective to direction using this measure.



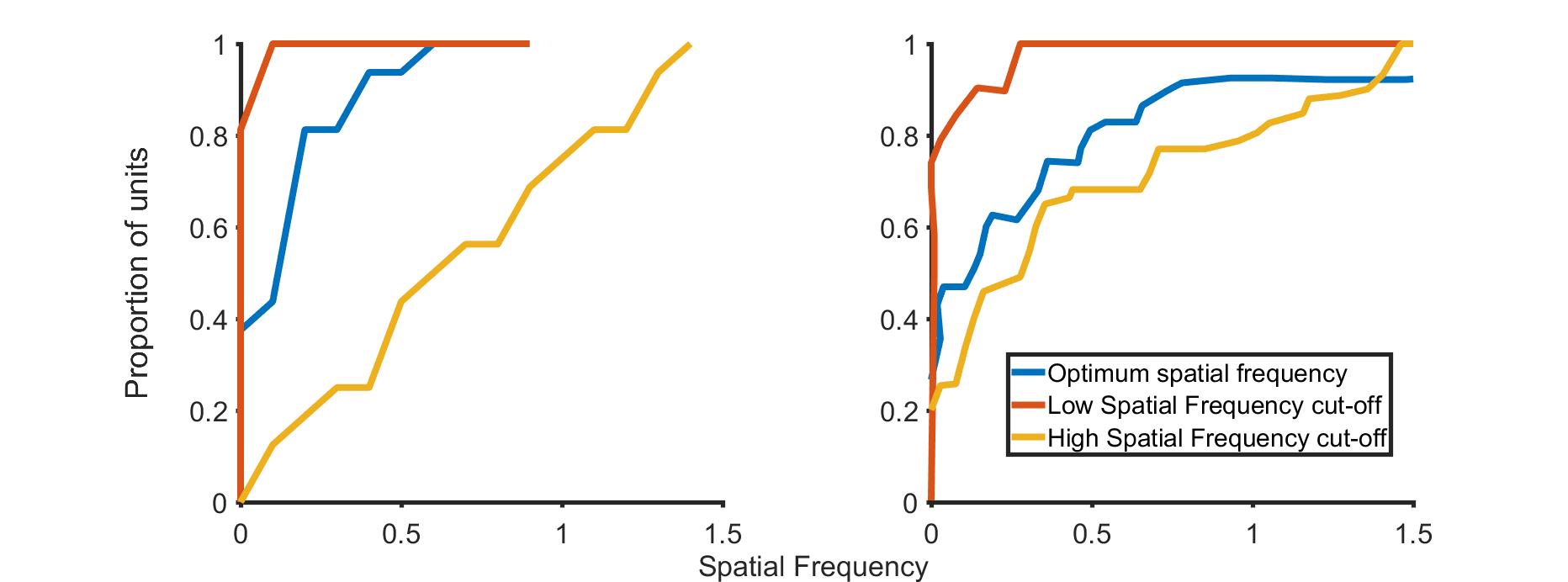
*Figure 5:* *Distribution of direction selectivity index of 22 superior colliculus neurons using two different measures: the DSI (a) and the DCV(b).*



*Figure 6:* Example of a cell that is selective to both direction and orientation (a) and a cell that is selective to direction but not orientation (b).

**Spatial Frequency Tuning**

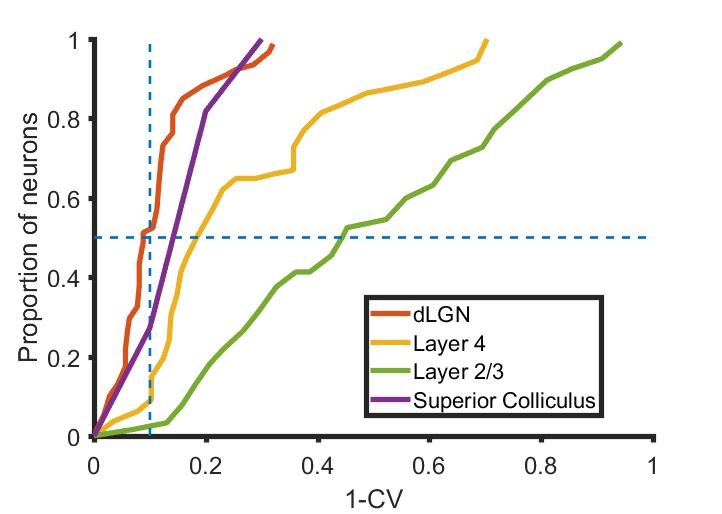
A summary of the results of spatial frequency tuning we obtained from 16 neurons is presented in figure 7a. The results from the LGN from Van Hooser et al., 2013, Figure 7b, 30 neurons) were plotted on similar axes in figure 7b to aid direct comparison. The median peak spatial frequency of the SC neurons was 0.2 cpd (95% CI= [0, 0.2]) and the median half width at half height was 0.35 cpd (95% CI= [0.15, 0.55]). SC neurons tended to have higher spatial frequency cut-offs when compared to the LGN neurons (figure 7b). A similar proportion of SC (80%) and LGN (76%) neurons were low-pass tuned to spatial frequency .



*Figure 7:* *Cumulative distribution of the low-cutoff, optimum and high cut off spatial frequencies in the tree shrew Superior Colliculus for 16 neurons (a) and the Lateral Geniculate Nucleus for 30 neurons (b; LGN). The LGN results were published in the paper by Van Hooser et al., 2013 (figure 7b) and plotted on the same scale as the SC data in the left hand side panel.*

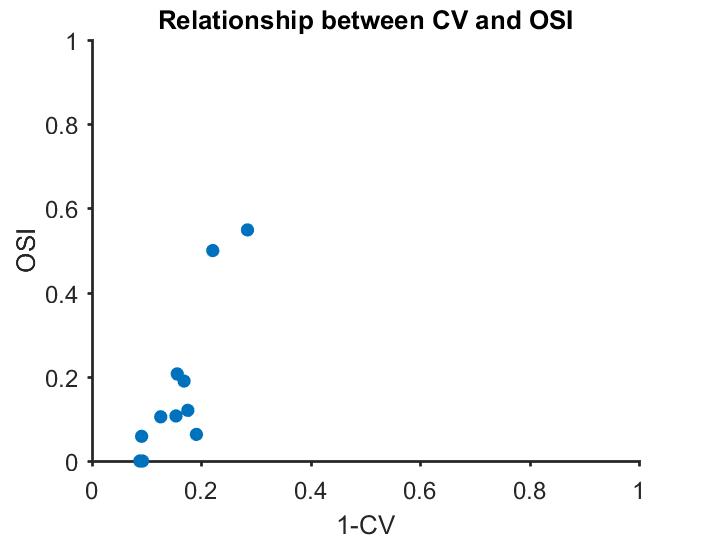
**Orientation tuning using gratings**

The circular variance of the orientation response of eleven neurons was calculated at the peak spatial frequency and compared with those of previously published data in the geniculostriate system of the tree shrews. The distribution of these circular variances are shown in figure 8. The median CV of SC neurons when measured using gratings was 0.84 (95% CI= [0.77 0.91]). The distribution of the CVs of the superior colliculus and the LGN were similar. While in the LGN nearly 50% were not tuned to orientation at the peak spatial frequency, only 30% of the SC neurons did not show orientation tuning (1-CV<0.1). None of the neurons demonstrated sharp orientation tuning (1-CV> 0.5) as observed in layer 2/3 of the cortex.

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*Figure 8: Comparison of the distribution of the orientation selectivity of the LGN, Layer 4 and Layer 2/3 neurons to the distribution of orientation selectivity of the superior colliculus neurons. Horizontal dotted line indicates the 50% of neurons and the vertical dotted line is the orientation tuning cut off.*

As there was only enough data in 11 neurons to enable circular variance calculations, the orientation selectivity index (OSI) was calculated for the 16 neurons, where gratings of both optimum and orthogonal orientation were calculated. The relationship of the OSI and the CV for the 11 neurons where both these data were available is shown in figure 9.

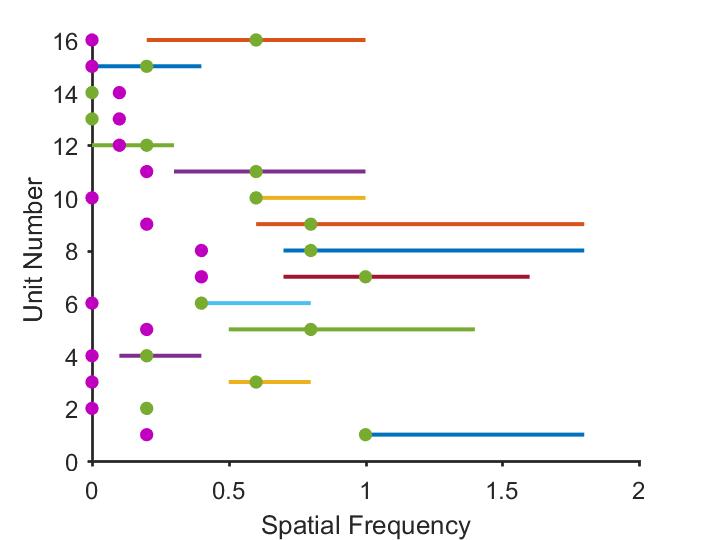


*Figure 9: Relationship between the circular variance and orientation selectivity index*

Levick and Thibos (1982) calculated the circular variance of the neurons in their study at different spatial frequencies and found that neurons showed sharper tuning at spatial frequencies higher than the optimum spatial frequency. Since the circular variance and orientation selectivity index show a strong correlation (r= 0.87, p<0.005, n=11), in this chapter, OSI was used to conduct a similar analysis.

**Relationship between spatial frequency and orientation tuning.**

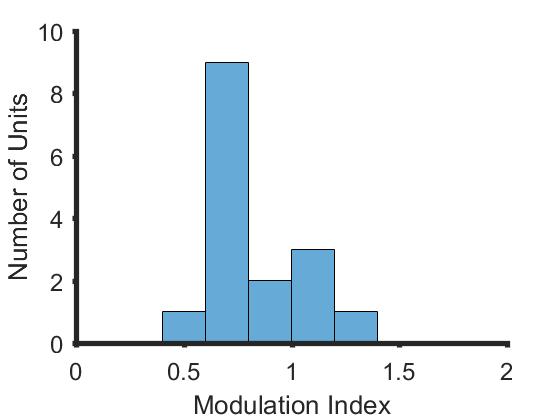
When the spatial frequency tuning response of the neuron at different orientations was measured, 13 of 16 neurons were tuned to orientation at higher spatial frequencies. The F0 components of a neuron’s response to gratings of increasing spatial frequencies at the optimum and the orthogonal orientations are shown in figure 1. The gray shaded area represents the spatial frequencies where the neuron still responds to gratings of the optimum orientation but no longer responds when gratings of the orthogonal orientation are presented (i.e., the neuron is orientation tuned). The difference in response between the optimum and non-optimum orientation cut off frequencies was calculated. These results for the group are presented in figure 10. A one tailed Wilcoxon Signed Rank test showed that the spatial frequency cutoff at the optimum orientation was significantly higher than the spatial frequency cutoff at the orthogonal orientation (median difference= 0.4 cpd; z=3.15; p=0.0008). The magenta circles in figure 10 show the peak spatial frequency of the respective neuron and the green circles, the spatial frequency where the neuron was most tuned to orientation (where the OSI was maximum). The spatial frequency where the orientation tuning of the neuron was greatest was significantly higher than the peak spatial frequency of the neuron (One-tail Wilcoxon Signed Rank test; z=3.3096; p=0.0005), indicating that orientation tuning was observed at higher spatial frequencies in the tree shrew SC.



*Figure 10:* *The difference between the cut-off frequencies for the optimum and orthogonal orientations for 16 units is shown in the above figure. The purple circles are the peak spatial frequencies of the respective neurons and the green circles are the spatial frequency where the cell is most tuned to orientation. In most cases, the neurons are most tuned for orientation at spatial frequencies well past the peak spatial frequency.*

**Modulation Index of the neurons.**

For the 16 units whose spatial frequency tuning were recorded, the distribution of modulation ratios is presented in figure 11. A modulation ratio less than one means that the modulated component of the response was lower than the unmodulated component (non-linear cells) while a modulation ratio of greater than 1 indicates that the neurons were linear. The median value of the modulation index was 0.76 (95% CI= [0.65, 1.03]) suggesting that most neurons in our sample showed non-linear summation over their receptive fields.



*Figure 11:* *The distribution of modulation indices of the neurons in our sample. Most neurons had a modulation index less than 1.*

**Discussion**

Our results show that the majority of neurons in the superficial layers of the tree shrew SC show orientation biases when tested with bar stimuli. When tested using grating stimuli, most neurons also showed low pass spatial frequency tuning and demonstrated orientation tuning at higher spatial frequencies. We also found that a small proportion of neurons were tuned to direction and the majority of neurons showed non-linear summation over their receptive fields.

We used bars to study the orientation selectivity of neurons in the superior colliculus and found that most units (90%) were biased for orientation. We calculated two measures of orientation selectivity the circular variance and bias. Neurons with a bias greater than or equal to 3 were comparable to the elongated receptive field units previously reported (Albano et al., 1978). Six out of twenty-two neurons (27 %) had a bias greater than 3. This is comparable to the proportion of elongated receptive field units (19%) that were reported by Albano et al (1978). Most of the elongated receptive field units reported by Albano et al (1978) were found in the lSGS and SO layers and none were recorded from the uSGS. In our sample, most neurons were biased for orientation to some degree and neurons that had a bias greater than 3 were found in both the upper and lower SGS with the sharpest tuned neurons found in the uSGS. We only recorded from one neuron in the SO which had a bias value of 2.85. This discrepancy in the detection of orientation biased neurons in the uSGS may be due to the thickness of the bar stimulus used.

Neurons in the subcortical areas show more tuned responses when shown thinner bars and at higher spatial frequencies (Vidyasagar and Urbas, 1982; Levick & Thibos, 1980; Levick & Thibos, 1982). In response to gratings, at the peak spatial frequency, the SC neurons showed minimal orientation biases and the orientation tuning of neurons were usually more evident at higher spatial frequencies. As a result, if Albano and colleagues used thicker bars than we have, they may have failed to find neurons that had elongated receptive fields in the uSGS.

When the spatial frequency tuning of SC neurons and the LGN neurons in the tree shrew were compared, both LGN and SC neurons showed low pass spatial frequency tuning. However, when compared to the LGN neurons (as published in Van Hooser et al., 2013), the peak spatial frequencies we observed were lower. This could be related to the type of neurons the SC neurons get their inputs from. SC neurons in most species receive inputs from achromatic Y-like or W-like cells (DeMonasterio, 1978; Shapley & Perry, 1986; Schiller & Malpeli, 1977; Bunt et al., 1975; Leventhal et al., 1981). These cells show non-linear summation over their receptive fields and tend to have lower peak spatial frequencies (Enroth-Cugell & Robson, 1966; Derrington & Fuchs, 1979; So & Shapley, 1981). Our results indicate that the SC neurons show non-linear summation (as Y and W cells do) over their receptive fields while the LGN neurons tended to show more modulated responses, typical of X-like neurons (Van Hooser et al., 2013). This could explain the difference in the peak spatial frequency of the neurons from the two sub-regiongs. One other difference between the LGN and the SC spatial frequency tuning curves was the high spatial frequency cut-offs. The SC neurons generally tended to have higher spatial frequency cut-offs compared to the LGN neurons. A possible reason could be that in our study, we carefully tested the responses of the neurons to stimuli of different spatial frequencies to ensure that the neurons could resolve gratings of higher spatial frequencies. If uncorrected refractive errors were present in the tree shrews in the study by Van Hooser and colleagues, then this could explain the difference in the high cut-off values reported between the LGN and the SC.

Most neurons in the tree shrew superior colliculus were not tuned to direction. Van Hooser et al reported that only one neuron in their entire sample was tuned to direction using DCV in the geniculo-striate system of tree shrews. Using the same measure, we found that none of our neurons exhibited direction selectivity. A larger sample may show a small proportion of neurons being tuned for direction using the DCV in the superior colliculus. In their study, Albano and colleagues reported that a proportion of the elongated receptive field cells were also selective to direction of movement and that these cells were found in the lSGS and the SO. However, the proportion of neurons tuned to direction was not reported. Using a less conservative measure than the DCV, the DSI, we found that approximately 20% of the neurons were tuned to direction. Of the 5 direction selective neurons, 2 were from the uSGS (13% of the uSGS neurons) and 3 were from the lSGS (50% of the lSGS neuons) suggesting that direction selective neurons were sparser in the uSGS when compared to the lSGS.

In their study, Albano et al., (1978) proposed that the uSGS and the lSGS could play different functional roles. They found that the uSGS neurons were composed of only one type of neuron (the stationary –responsive type) while the lSGS consisted of a combination of different type of neurons. This claim was supported by anatomical and morphological evidence; namely, uSGS neurons were generally smaller (5-8 μm) and received predominantly retinal inputs and lSGS neurons were larger (8-12 μm) and received inputs from both the retina and the cortex (Abplanalp, 1971). A later study however showed that the retinal inputs dominated the SGS of the tree shrews with the uSGS receiving less cortical inputs than the lower SGS. (Graham & Casagrande, 1980). We found that the uSGS contained the sharpest tuned neurons in our sample and a smaller proportion of these neurons were tuned to direction whereas, all the neurons in the lSGS were broadly tuned to orientation and a higher proportion of neurons were tuned to direction. When compared to Albano et al., we found more diverse response properties in the uSGS rather than the lSGS (e.g.: see figure 4c). However, our sample size in the lSGS was small (6 uSGS neurons) and any lack of diversity in the neuronal responses could be attributed to sampling errors.

In this study we found that neurons in the superior colliculus were tuned to orientation at higher spatial frequencies. Most neurons were low pass tuned to spatial frequency and at the peak spatial frequency, a similar proportion of neurons were biased for orientation in the LGN as well as the SC. In the SC, neurons were sharply tuned to orientation at higher spatial frequencies. This result is consistent with that observed in the retina and the LGN of cats and macaques. Layer 4 neurons of the tree shrews, which resemble LGN neurons (Van Hooser et al., 2013), also respond similarly at higher spatial frequencies (See Chapter 5). This similarity in subcortical orientation response across both the geniculo-striate system as well as the SC indicate that orientation biases observed in the LGN and the SC could be inherited from the retina. In the tree shrew then, sharp orientation tuning observed in the supra-granular layers could be due to the sharpening of orientation biases by intra-cortical inhibition rather than through Hubel and Wiesel like excitatory convergence.

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