GRANT PROPOSAL

Mechanisms underlying the development of orientation maps,

pinwheels and singularities in cat area 18.

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SPECIFIC AIMS

The long-term goal of this research is to identify the developmental basis of the intricate map of receptive fields observed in the adult mammalian visual cortex. The various receptive field properties--retinotopic location, ocular dominance, orientation selectivity, spatial frequency tuning--must all be fit onto a two-dimensional cortical surface so that uniform coverage is provided. Understanding the developmental mechanisms which establishes this organization is an important task for developmental neurobiology.

The short-term objective is the identification the specific factors associated with the development of the map of orientation selectivity. Advances in optical imaging have revealed an intricate pattern of orientation selective neurons in the primary visual cortex consisting of iso-orientation domains which change smoothly over most of the cortex but change abruptly at discrete locations, or singularities. The orientation map varies radially around the singularities through 180 degrees of orientation, forming a pinwheel-like pattern. Both the origin and function of the singularities are open questions.

The singularities may represent a side-effect of the developmental interactions that create the orientation map. Consider the problem of projecting the surface of the earth onto a two dimensional map. In order to not distort the continents, discontinuities need to be introduced in the form of gaps in the surface, usually placed in the middle of oceans. If, however, all of the continents were concentrated in a small region of the globe, projecting the surface could be done with fewer discontinuities.

Analogously, the singularities may be discontinuities needed in order to fit all of the receptive field properties on a two dimensional surface. If this is the case, we might expect that reducing the number of represented orientations will reduce the number of singularities.

On the other hand, the singularities may be the product of developmental processes distinct from the processes that create the rest of the orientation map. If this is the case, then reducing the number of represented orientations would not be likely to reduce the number of singularities.

The specific aim of this research is to determine the factors which influence the development of the orientation maps and the development of the singularities, treating the two as potentially distinct phenomenon. Throughout this proposal, the term "orientation map" will refer specifically to the smoothly varying regions of these maps, while "singularity" will refer to the rapidly changing regions.

BACKGROUND AND SIGNIFICANCE

Hubel and Wiesel first described both the system of ocular dominance columns and the system of orientation columns while studying receptive field organizations in the cat primary visual cortex (Hubel and Wiesel, 1974). The systematic change in orientation selectivity along trajectories tangential to the cortical surface led them to hypothesize that the two maps formed orthogonal sets of columns. Such an orthogonal arrangement, where the orientation columns intersect the ocular dominance columns at right angles, would provide uniform retinotopic coverage of both ocular dominance and orientation selectivity.

Developmental mechanisms influencing the appearance of the ocular dominance columns have been studied extensively. Monocular deprivation experiments have demonstrated that the formation of ocular dominance columns for one eye depends on patterned visual input to that eye. If patterned input is deprived by suturing the eye, the width of that eyes ocular dominance columns decreases accordingly. However, reversing the deprivation during an early critical period allows the deprived eye to recover the lost territory. This implies that an activity-dependent competition is responsible for the formation of the ocular dominance columns (LeVay et. al. 1980).

Developmental mechanisms underlying the formation of the orientation columns have also been tested. Monocular deprivation results in a loss of orientation selectivity for neurons driven by the deprived eye. Like ocular dominance columns, reverse occlusion allows recovery of the original orientation map (Kim and Bonhoeffer, 1994).

Binocular deprivation reduces but does not prevent formation of the orientation maps in ferret (Chapman and Stryker, 1993). This experiment, however, was based on electrophysiological recordings of orientation selectivity, which does not capture the pinwheels and singularities. In order to test for the effect of binocular deprivation on pinwheels and singularities, this experiment is proposed here using optical imaging techniques described below.

TTX blockade of the visual cortex prevents formation of the orientation maps, suggesting that the orientation maps, like the ocular dominance columns, is an activity-dependent process (Chapman and Stryker, 1993). However, the formation of orientation maps following TTX blockade of the retina has not yet been reported. This experiment is proposed here.

Further experiments have been undertaken to determine whether the orientation maps are experience-dependent. These experiments restricted the visual environment of kittens to single orientations. The result was a shift in the orientation columns in favor of the experienced orientation at the expense of the inexperienced orientation (Singer et. al. 1981).

The development of electrically sensitive dyes and their use in imaging cortical activity over the last several years has yielded a striking picture of orientation maps. As mentioned above, the maps form singularities and pinwheels rather than forming a system of regular slabs like the ocular dominance columns (Blasdel 1992). The developmental mechanisms underlying this pattern is the subject of current research, including this grant proposal.

The animal model for the current experiments will be the cat. Demonstrations of pinwheels and singularities in area 18 of the cat visual cortex (Bonhoeffer and Grinvald, 1991) have made the cat an ideal choice for this type of experiment. Access to area 18 is fairly straightforward. The ability to image activity over long periods of time requires a certain degree of stamina from the subject, implying that a larger animal makes a better subject for these types of experiments. Also, effective resolution is increased by a larger cortical area. Primates, however, would be unnecessary for these experiments, as the recordings are not being made from behaving animals.

EXPERIMENTAL DESIGN AND METHODS

Binocular TTX Blockade Experiment

The techniques for this experiment are modified from Stryker and Harris (1986).

Subjects

Eight kittens will be used as experimental subjects and three kittens as controls in this experiment.

Methods

5 mM TTX in citrate buffer will be injected into the vitreous humor of the experimental subjects, once every 3 days. Initially, a 1 microliter injection will be made. This injection will be increased by 5-10% for each injection. The injections will be made with an 18 mm 30 gauge needle with a flange 3 mm from the tip to prevent insertion to far into the eye. This is important because the TTX dose is greater than a lethal dose, if inadvertently injected into the bloodstream.

The kittens will be anesthetized with halothane for the injection. The injection site is at the lateral margin of the eye, posterior to the ora serrata. A small incision made in the lateral conjunctivum exposes the site. To ensure the needle does not hit a blood vessel, it needs to be inserted once and then withdrawn. The injection is made at that site if no blood is observed during this initial insertion. The incision is closed with a wound clip, and then the procedure is repeated for the other eye.

The control kittens undergo the same procedure, only using a 5 mM citrate buffer solution with no TTX.

The injections will begin 14 days after birth and continue to 6 weeks of age. At 6 weeks of age, an orientation map will be recorded for control and experimental animals based on the general methods described below.

Analysis

The orientation maps will be analyzed as described in the general methods below. The area of orientation selectivity is calculated for all animals, as well as singularities per unit area of orientation selectivity. If the area of orientation selectivity is very small, the singularities per unit area will not be reliable.

Averages of these values for the control and experimental animals are tabulated.

Interpretations

If the average area of orientation selectivity for the TTX-blockade is significantly lower than in the control, then the development of the orientation maps requires at least retinal activity. If the singularities per unit area orientation selectivity is significantly lower in the TTX blockade animals than in the control, then the development of the singularities requires at least retinal activity.

To determine whether the development of the orientation maps and the singularities is experience dependent, the results from this experiment will need to be compared to the results of the binocular deprivation experiment.

Binocular Deprivation Experiment

The techniques for this experiment are modified from Chapman and Stryker (1993).

Subjects

Ten kittens will be used as experimental subjects and three as controls in this experiment.

Methods

2 week old kittens will be anesthetized with halothane for the lid-suturing surgery. For both eyes, the lids are pulled apart and the lid margins excised. The lids are then sutured together and the animals returned to their mothers. Sutures are removed 7 days after surgery, at which point the two lids should have fused. Visual inspection will be used to ensure that the lids have not come apart--if so, the animals will not be used in this experiment. Binocularly sutured animals are raised in a normally lit environment.

The control kittens are anesthetized with halothane at 2 weeks of age. The eye lids are gently pulled apart, and then the animals are returned to their mother.

At six weeks of age, orientation maps are constructed for both experimental and control subjects. This procedure is described in the general methods below.

Analysis

The orientation maps will be analyzed as described in the general methods below. The area of orientation selectivity is calculated for all animals, as well as singularities per unit area of orientation selectivity. Averages of these values for the control and experimental animals are tabulated.

Interpretations

If the average area of orientation selectivity for the binocular deprivation is significantly lower than in the control, then the development of the orientation maps is not only activity dependent, but also experience-dependent. If the singularity density is significantly lower in the binocularly deprived animals than in the control, then the development of the singularities is presumably experience-dependent.

Comparing the results of this experiment with the previous experiment should begin to determine whether the development of the orientation maps occurs by a different mechanism than the development of the singularities. For instance, if singularities were found to be activity-dependent but not experience-dependent, while the orientation maps were found to be experience-dependent, this would imply that the singularities were not merely side-effects of the development of the orientation maps.

Single Orientation Experiment

Methods for this experiment are partially derived from Singer, Freeman, and Rauschecker (1981).

Subjects

Ten kittens will be used as experimental subjects and three as controls in this experiment.

Methods

Kittens will be fitted with goggles from the time of eye opening until six weeks of age. The goggles consisted of cylindrical lenses with a 2 cm diameter. The lenses have a -25 dptr refractive power for one axis and 0 dptr for the orthogonal axis. These lenses effectively attenuate orientations with greater than 10 degrees difference from the 0 dptr axis, effectively limiting visual experience to one orientation. Previous studies indicate that these lenses do not significantly affect binocularity (Rauschecker and Singer, 1981).

The kittens are then raised in well-lit boxes whose walls were painted with contrast contours at all orientations. Previous studies have raised kittens with no goggles in boxes with only single-orientations painted on the walls. This can not assure exposure to limited orientations, as the kittens movements may alter the range of experienced orientations. The present experimental design provides a more complete control over the actual orientations experienced by the kittens.

Control kittens were raised in boxes with contrast patterns at all orientations from the time of eye opening until six weeks of age, but with no goggles.

At six weeks of age, orientation maps are constructed for both control and experimental animals following the general methods described below. The goggles are removed from experimental animals before mapping orientation selectivity.

Analysis

The orientation maps will be analyzed as described in the general methods below. The area of orientation selectivity is calculated for all animals, as well as singularities per unit area of orientation selectivity. In addition, the orientation map is subdivided into regions selective for orientations orthogonal to the lens axis (inexperienced orientations) as well as regions selective for orientations parallel to the lens axis (experienced orientations). The areas of the experienced and inexperienced areas are calculated as well.

Interpretations

If the area of experienced orientations is greater than the area of inexperienced orientations, the development of the orientation map is dependent on the actual orientations present in the visual field. This result would be predicted based on Singer et. al. (1981).

If the singularity density is significantly lower in experimental animals than in control animals, then the development of singularities is dependent on the actual orientations present in the visual field. This would support the hypothesis that singularities are a side effect of the development of orientation maps. If the singularity density is significantly higher or not significantly different between experimental and control situations, this would imply that singularities are the result of distinct developmental mechanisms.

General Methods for Mapping Orientation Selectivity

These procedures are modified from Blasdel (1992).

Animals are anesthetized with sodium pentabarbitol and paralysed with succinyl choline-HCl. The head is secured with a stereotaxic frame. The scalp is reflected, and a trephine is used to bore a 20 mm hole on the cranium superficial to area 18. A stainless steel chamber is then cemented to the cranium. The chamber is sealed with a Teflon plug until the time of recording.

For optical recording, the dura within the chamber is deflected and a plug with a glass window is inserted and adjusted to less than a millimeter above, but not touching, the cortical surface. Two tubes attached to the plug allows the dye solution (NK2367, 0.1% in saline) access to the cortex.

The cortex is illuminated by a 100W tungsten halogen lamp directed through a heat filter and an interference filter (720 nm, to maximize NK2367 signal) into a fiber optic cable. The light from the cable is collimated with a prism and directed onto the cortex through the edge of a 50 mm objective lens. Optical activity is reflected back through this same lense and projected onto a CCD camera for digitization, storage, and processing.

To test record orientation selectivity, the kittens were mildly paralyzed with an injection of pancuronium or vecuronium bromide to stabilize the eyes. The head is secured in a stereotaxic frame. A 19 inch monitor (60 Hz) positioned 1.5 m from the eyes is used to display stimuli.

The stimuli consist of full-field oriented lines consisting of non-harmonic spatial frequencies of a single orientation. The non-harmonic frequencies, which contain components at all spatial frequencies, control for possible frequency selectivity of individual units.

The time course for recordings is as follows. The cortex is allowed to stabilize for approximately 30 seconds. During this time, the animal sees a blank gray screen. At the end of this interval, frames from the CCD are collected for 1-3 seconds, averaged, and stored in a frame buffer.

Then, the stimulus at a particular orientation is displayed. After a delay of 0.5-1.0 second, frames from the CCD are collected for 1-3 seconds, averaged, and stored in a second frame buffer.

The screen is again blanked for approximately 20 seconds. Then frames are collected for 1-3 seconds, averaged, and stored in a third frame buffer.

Finally, a stimulus at the orthogonal orientation to the first stimulus is displayed. After a 0.5-1.0 second delay, frames are collected for 1-3 seconds, averaged, and stored in a fourth frame buffer.

The exact time interval over which frames are averaged can vary, depending on vascular activity. Previous studies indicate that vascular activity begins 2-3 seconds after activation of a particular area. No effective means of correcting for this activity have been devised, so the duration of frame collection and averaging is adjusted between 1 and 3 seconds in order to maximize signal/noise ratio while minimizing vascular activity-induced artifacts.

For any particular pair of orthogonal orientations, this sequence of four collection steps are repeated three to ten times and averaged off-line, yielding four composite frames corresponding to the four collection steps. Orientations are stepped by 15 degrees, so complete coverage of all orientations is achieved after six steps.

Analysis

The preferred orientation at a particular cortical position is calculated as follows. For a particular orientation, the first composite frame is used to establish a baseline of activity. The difference in activity between the second and first composite frames is used to estimate the magnitude of response at that orientation. Similarly, the third frame establishes a baseline for the orthogonal orientation, and the difference in activity is used to estimate the magnitude of response at the orthogonal orientation.

To combine these two response magnitudes, the angles of the original and orthogonal orientations are multiplied by two. This is because the orientations, being unsigned quantities, vary from 0 to 180 degrees and opposing orientations differ by 90 degrees. If the orientations are transformed so that they vary from 0 to 360 degrees, orthogonal orientations will then differ by 180 degrees. The different responses can be vectorially summed using the response magnitude as the vector modulus and the transformed orientation as the vector angle. In this way, equal responses of a unit to two orthogonal orientations will cancel.

This same method allows responses from each of the six orientation increments to be combined to yield a single estimate of the unit's preferred orientation. These estimates can be compiled across all cortical locations to create a graphical representation of the orientation map by assigning a different color to each of the transformed orientations.

The result is a color depiction of a vector displacement field for the area of cortex recorded from. The displacement field should change slowly and continuously for most of the cortex, but rapidly in the vicinity of singularities.

The displacement can be used to determine the area of orientation selectivity by simply processing with a threshold function. The result is a field which is zero at all orientation non-selective locations and positive at all orientation selective locations. Integrating this field over two dimensions yields the area of orientation selectivity, which can used to calculate the percentage of observed cortical region which is orientation selective.

To quantify singularities, the second spatial derivative of the displacement field is taken and then processed with a threshold function. The result is a field which is non-zero at the singularities and zero everywhere else. To count the number of singularities, one need only count the number of contiguous non-zero regions. This singularity count can be used to estimate the degree of development of singularities. However, because singularities may be present without sufficient orientation selectivity to be observable, it is more useful to divide the number of singularities by the area of orientation selectivity and use this as an estimate of singularity density.

Timetable

Year 1 Year 2 Year 3

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TTX Blockade Exp X

Binocular Deprivation Exp X X

Single Orientation Exp X X

REFERENCES

Blasdel GG (1992) Orientation Selectivity, Preference, and Continuity in Monkey Striate Cortex. J Neurosci 12(8):3139- 3161.

Bonhoeffer T, Grinvald A (1991) Iso-orientation domains in cat visual cortex are arranged in pinwheel-like patterns. Nature 353:429-431.

Chapman B, Stryker MP (1993) Development of Orientation Selectivity in Ferret Visual Cortex and Effects of Deprivation. J Neurosci 13(12):5251-5262.

Hubel DH, Wiesel TN (1974) Sequence regularity and geometry of orientation columns in the monkey striate cortex. J Comp Neurol 146:421-450.

Kim D, Bonhoeffer T (1994) Reverse occlusion leads to a precise restoration of orientation preference maps in visual cortex. Nature 370:370-372.

LeVay S, Wiesel TN, Hubel DH (1980) The development of ocular dominance columns in normal and visually deprived monkeys. J Comp Neurol 191:1-51.

Rauschecker J, Singer W (1981) The effect of early visual experience on the cat's visual cortex and their possible explanation by Hebb synapses. J Physiol (London) 310.

Singer W, Freeman B, Rauschecker J (1981) Restriction of Visual Experience to a Single Orientation Affects the Organization of Orientation Columns in Cat Visual Cortex. Exp Brain Res 41:199-215.

Stryker MP, Harris WA (1986) Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. J Neurosci 6(8):2117-2133.