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No _{ON-OFF} Maps in Supragranular Layers of Ferret Visual Cortex

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Abstract

Primary visual cortex contains functional maps of a number of stimulus properties including ocular dominance, orientation, direction, color, and spatial frequency. These maps must be organized with respect to each other and to a single continuous retinotopic map of visual space such that each stimulus parameter is represented at each point in space. In the ferret, geniculocortical inputs to cortical layer IV are segregated into _{ON-} and _{OFF-}center patches, suggesting the possibility that there might be an additional cortical map in this species. We have used optical imaging of intrinsic signals to search for _{ON-OFF} maps in ferret visual cortical cells and have found none. This suggests that the high degree of _{ON-OFF} segregation seen subcortically in the ferret may play a role in the development of visual cortical receptive fields rather than in adult cortical function.

INTRODUCTION

Within the mammalian visual system, cells with different receptive field properties are often segregated anatomically. How such segregation develops and whether it has functional consequences in the adult are important questions. In the ferret, the _{ON} and _{OFF} channels (signaling increments and decrements of light respectively) are not only segregated in the retina as in other species but are also segregated into separate _{ON} and _{OFF} sublaminae in the lateral geniculate nucleus (LGN) (Stryker and Zahs 1983) and into _{ON} and _{OFF} patches of LGN afferents in cortical layer IV (Zahs and Stryker 1988). If this uneven spatial distribution of information was maintained throughout the cortical layers, then ferrets would have an additional map of _{ON} versus _{OFF} inputs that would have to be superimposed on the retinotopic map along with maps of other visual stimulus properties such as ocular dominance, orientation, direction, and spatial frequency (for review, see Swindale 2000). The constraint imposed by such additional spatial organization (Swindale 2000) might explain the rather odd layout of ferret visual cortex including large and irregular ocular dominance columns and visuotopic discontinuities (White et al. 1999).

METHODS

Adult female ferrets were prepared for optical imaging as follows: anesthesia was induced using acepromazine (0.04 mg/kg im) and ketamine (40 mg/kg im). Atropine (0.1 mg/kg) was injected subcutaneously. Animals were intubated and respired and anesthesia was maintained using 1–2% isoflurane. Ferrets were placed in a stereotax on a heating pad and rectal temperature and EKG were monitored. A craniotomy was performed over left area 17, and the dura was retracted. Agar (2%) and a glass coverslip were applied over the craniotomy. Atropine and neosynephrine eye drops were used. Animals wore contact lenses to focus the eyes on the monitor (viewing distance, 33 cm). Visual stimuli in the first experiment were alternating 3-s presentations of a black or white screen. In the second experiment, the screen changed from

black, to dark gray, to medium gray, to light gray, to white (or vice versa) in four equal steps of luminance, each presented for 600 ms. In the final experiment, visual stimuli consisted of drifting square-wave gratings (drift velocity, $10^\circ/\text{s}$; spatial frequency, $0.5 \text{ cycles}/^\circ$) presented at four different orientations. In this experiment, after normal orientation maps were obtained, ON-center activity blockade was induced by binocular injection of $\text{DL-2-amino-4-phosphonobutyric acid}$ (APB) to obtain a vitreal concentration of $700 \mu\text{M}$ (Chapman and Gödecke 2000). Specific ON-center blockade of retinal activity in both eyes was confirmed by electrophysiological recording in the right LGN (Fig. 2A). After the blockade was established, orientation maps were again collected. Optical imaging was performed using the ORA 2001 system (Optical Imaging). First-frame analysis was used to minimize blood vessel artifacts (Bonhoeffer and Grinvald 1996; Grinvald et al. 1986). All procedures were approved by the University of California, Davis Animal Care and Use Committee, and were in accordance with the National Institutes of Health Guidelines.

RESULTS

We used optical imaging of intrinsic signals (Bonhoeffer and Grinvald 1996; Grinvald et al. 1986) to look for ON-OFF maps in ferret visual cortex. In preliminary experiments in four adult ferrets, we used alternating full-field light or dark screen stimuli and calculated light versus dark activity maps. No ON-OFF patches were seen in such maps (Fig. 1A). Because cellular responses to light and dark flashes tend to be relatively transient, whereas changes in optical imaging signals (mostly blood oxygenation state) are relatively slow (Bonhoeffer and Grinvald 1996), we next tried using full-field stimuli that gradually darkened or lightened in several steps during the stimulus presentation in another four animals. Again no ON-OFF patches were found (Fig. 1B). Other activity maps such as orientation and ocular dominance were completely normal in all eight animals.

The lack of ON-OFF maps seen in these experiments could be due to cortical cells not responding well to the full-field stimuli. Therefore we next took a different approach, taking advantage of the ON-center retinal activity blockade produced by APB (Slaughter and Miller 1981). For these studies, we used oriented square-wave grating stimuli to which cortical cells respond very well. After recording normal orientation maps in six adult ferrets, APB was injected binocularly to produce an ON-center activity blockade (Chapman and Gödecke 2000) (Fig. 2A). New orientation activity maps were then collected (Fig. 2B). Two different methods were then used to look for ON-OFF maps. First, the sums of the orientation maps before APB were compared with those after APB. The difference between the sum before APB (where both ON and OFF activity are present) and the sum after APB (where only OFF activity is present) should reveal any ON-OFF segregation. This method is analogous to the way that ocular dominance maps are generally calculated by comparing the sum of orientation maps from one eye with the sum of maps from the other eye. In fact, the difference maps comparing activity before and after APB showed no ON-OFF patches, indicating a lack of segregation (Fig. 3A). Second, individual orientation maps before and after the APB treatment were compared. The maps with only OFF activity present appeared very similar to the normal maps with both ON and OFF activity (Fig. 2B). The degree of similarity of the maps seen before and after APB injections was quantified using cross-correlation. The degree of similarity seen in the APB-treated animals was statistically indistinguishable from that seen in control animals treated with binocular saline injections (Fig. 3B), again indicating the lack of ON-OFF maps.

DISCUSSION

Our data show that although ON and OFF geniculate afferents to the cortex are anatomically segregated in layer IV, the two channels are mixed by the level of the supragranular layers [which contribute most of the optical imaging signal (Bonhoeffer and Grinvald 1996)]. The

loss of _{ON} and _{OFF} segregation somewhere between the afferent input to the cortex and the output layers suggests that the segregation of _{ON} and _{OFF} LGN afferent inputs to cortex plays no role in higher cortical function in the adult animal. However, it is possible that the _{ON-OFF} segregation could function in setting up the response properties of layer IV cells. We are currently studying receptive field structure in layer IV cells of ferret cortex, and preliminary data suggest that the majority of these cells do receive both _{ON} and _{OFF} inputs but that there are some cells receiving only one type of input, indicating that at least most _{ON-OFF} segregation is already lost at the level of layer IV cells (Usrey and Chapman 2001). It is likely that the segregation of geniculocortical afferents, like the segregation of _{ON}- and _{OFF}-center cells into different sublaminae in the LGN occurs during development by an activity-dependent process (Cramer and Sur 1997) driven by the correlation structures of _{ON} and _{OFF} center activity in retinal ganglion cells (Wong and Oakley 1996). Such activity-dependent processes may be necessary for the development of important functional properties such as simple-cell orientation selectivity (Miller 1994), and _{ON-OFF} segregation may be a byproduct of these processes. Computational modeling has shown that variations in the exact patterns of activity during development can lead to variations both in the degree of anatomical segregation of _{ON} and _{OFF} information and in the percentage of layer IV cells that are orientation selective (Miller 1994). Minor changes to the model parameters describing correlations in _{ON} versus _{OFF} activity can lead to both _{ON-OFF} segregation and decreased orientation selectivity as is seen in the ferret (Chapman and Stryker 1993; Welicky and Katz 1997) or to lack of segregation and increased orientation selectivity as seen in the cat.

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FIG. 1. Lack of ON-OFF maps

A: the difference between $_{ON}$ and $_{OFF}$ activity maps for 2 of 4 animals where visual stimuli consisted of alternating black or white full-field flashes. Similar results were seen in the other 2 animals. **B:** the difference between $_{ON}$ and $_{OFF}$ activity maps for 2 of 4 animals where visual stimuli consisted of stimuli that went from white to black or from black to white in 4 equal steps during each stimulus presentation. Scale, 1 mm.

FIG. 2. Activity before and after binocular DL-2-amino-4-phosphonobutyric acid (APB) injections
A: intravitreal APB injections silence ON-center activity in ferret lateral geniculate nucleus (LGN) while leaving OFF-center activity intact. *Top:* peristimulus time histograms (PSTHs) showing multicellular ON-center responses recorded in LGN lamina A. Normal response before eye injection. (*left*); lack of response at the same location after the injection of APB sufficient to produce a vitreal concentration of 700 μ M APB into the contralateral eye (*right*). *Bottom:* PSTHs showing multicellular OFF-center responses recorded in LGN lamina A. Normal response before eye injection (*left*); unchanged response at the same location after the injection of APB into the contralateral eye (*right*). Similar data were collected for ipsilateral eye injections. **B:** optical imaging orientation activity maps recorded in visual cortex of 1 of 6 ferrets. *Top:* normal

maps in response to 4 different orientations of moving square-wave gratings. *Bottom*: maps 30 min after binocular 700 μ M APB. Scale, 1 mm.

FIG. 3. Comparison of maps before and after silencing retinal on-center activity

A: ON plus OFF vs. OFF activity maps for 4 of 6 animals treated with binocular 700 μ M APB. Similar results were seen in the other 2 animals. Scale, 1 mm. **B:** quantification of map similarity (Chapman et al. 1996) for preand post injection maps for 6 animals treated with binocular 700 μ M APB and 4 control animals treated with binocular saline. Histogram shows the mean correlation coefficient, bar shows SD.