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Short communication

Blockade of afferent impulse activity disrupts on/off sublamination in the ferret lateral geniculate nucleus

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Abstract

The ferret retinogeniculate projection is organized into on/off sublaminae within each eye-specific layer. The role of afferent activity in the formation of these sublaminae was examined. We report that when the activity of retinal ganglion cells is blocked with tetrodotoxin during postnatal weeks three and four, on/off sublamination is disrupted, supporting a role for afferent activity in the formation of this pattern.

Keywords: Retinogeniculate; Development; Tetrodotoxin; Pattern formation; Retinal ganglion cell; Spontaneous activity

Electrical activity is considered to play a crucial role in the fine-tuning of connections in the mammalian brain [7]. Perhaps the most extensive evidence for a role for electrical activity in pattern formation in mammalian nervous system development comes from experiments on segregation of thalamocortical afferents into ocular dominance columns in primary visual cortex of cats. Reducing afferent activity from both eyes by neonatal lid suture (see [22] for review), or blocking retinal activity entirely by intraocular injection of tetrodotoxin (TTX) [24], prevents the normal segregation of geniculate afferents into ocular dominance columns in cortex [1]. Suturing only one eye neonatally causes an acute imbalance in activity from the two eyes reaching cortex; as a consequence, geniculocortical afferents from the open eye are larger than normal while those from the closed eye are smaller [19,20]. Activity in not only the presynaptic afferents but also in postsynaptic target neurons is critical for the development of geniculocortical connections. Reducing postsynaptic activity by blocking NMDA receptors with intracortical infusion of d-APV prevents the plasticity of thalamocortical connections after monocular lid suture [3]. Moreover, silencing postsynaptic cortical cells entirely by infusion of the GABAa agonist muscimol actually causes geniculocortical arbors related to the closed eye to be larger [13]. Thus, pre- and postsynaptic activity together regulate the rearrangement of geniculocortical arbors and the formation of synaptic connections during development of visual cortex.

In contrast, the evidence for a role for electrical activity in retinogeniculate development is rather less established. Infusing TTX into the vicinity of the optic tract in fetal cats appears to prevent the segregation of afferents from the two eyes into eye-specific layers [21], but this manipulation nonspecifically silences both presynaptic retinal afferents and postsynaptic LGN cells. In ferrets, retinogeniculate afferents segregate first into eye-specific layers and subsequently into on-center and off-center sublayers [12]. When NMDA receptors on postsynaptic LGN cells are blocked during the period of sublaminar segregation, retinogeniculate afferents fail to form sublaminae [12]. Whether or not afferent activity by itself is required for retinogeniculate pattern formation remained without critical examination. We now show that blocking retinal afferent activity specifically with intraocular injections of TTX prevents the segregation of retinogeniculate terminations into on/off sublayers in ferrets. Together with the ocular dominance system in visual cortex, then, the on/off system in the LGN provides compelling evidence for the involvement of pre- and postsynaptic activity in pattern formation in the visual pathway.

Retinogeniculate afferents in ferrets segregate into on/off sublayers approximately between postnatal day (P) 14 and P25 [8,12]; thus retinal activity blockade in this study spanned this period of development. Intraocular

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injections of TTX or neuronal tracers were performed under ketamine anesthesia (30 to 40 mg/kg, intramuscular), in some cases supplemented with inhaled metofane. The left eyelid was opened and a small hole was made in the vitreal chamber using a 27 gauge needle. Injections were made through this hole using a Hamilton syringe. In a preliminary experiment, we verified that an intraocular injection of 1 mg TTX (1 mg/ml in acetic acid buffer) in an adult ferret was sufficient to inhibit pupillary constriction in both the injected and the contralateral eye in response to light. We estimated that the vitreal volume in two week old ferrets was about one third that of the adult. We thus injected 0.3 μg TTX into the left eye on P12, and gave an additional injection every three days, increasing the dose by 10% with each injection. The last intraocular injection was on P24 and contained, in addition, 10 μl wheat germ agglutinin coupled to horseradish peroxidase (WGA-HRP) in order to label the projection from the left eye to the LGN. Control animals received five intraocular injections containing only acetic acid buffer on the same schedule as the experimental animals; the final intraocular injection included 10 μl WGA-HRP. In some experiments,

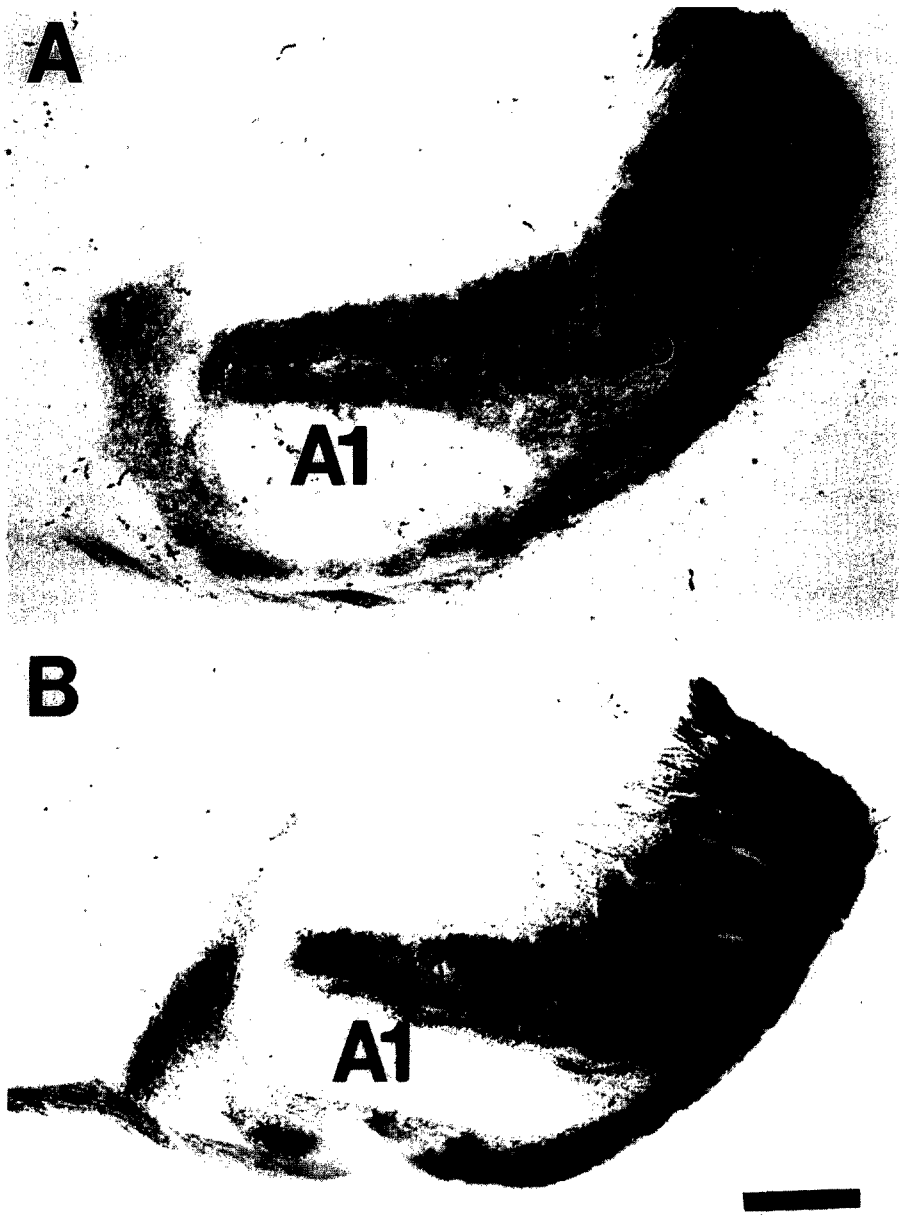

Fig. 1. A: horizontal section through the LGN of a normal P26 ferret following anterograde labeling of retinal projections from the contralateral eye with WGA-HRP. The A layer is divided by a pale staining intersublaminar zone, and the inner (A_i) and outer (A_o) sublaminae can be discerned. The ipsilateral recipient A1 layer is indicated. B: horizontal section through the LGN of a P26 ferret that had been treated with 5 intraocular injections of TTX prior to anterograde labeling with WGA-HRP. Staining in the A layer is uniform, and no division between inner and outer sublaminae is evident. Scale bar, 200 μm, applies to both panels.

animals received only 2 intraocular injections of TTX, containing 0.5 to 1 μg each, on P14 and P19. These animals were given an additional injection of 10 μl WGA-HRP on P24. Normal animals were also included in this study; these animals received only an intraocular injection of 10 μl WGA-HRP on P24. Animals were perfused on P26 with saline and 4% paraformaldehyde. Brains were immersed in 30% sucrose with 0.5% paraformaldehyde for several days, sectioned at 50 μm in the horizontal plane using a freezing microtome, and processed using tetramethylbenzidine to reveal HRP [16].

To assess the effect of activity blockade on retinogeniculate sublamination, the projection patterns to the LGN were scored blind, i.e., the observer did not know from which treatment group the histological sections were taken. Each section was given a score from 0 to 3, based on the extent of the labeled A layer that was visibly subdivided by a staining density minimum in the intersublaminar region that bisected the A layer [8]. A score of 0 signified no sublamination in the A layer, a score of 3 signified complete sublamination in the A layer, and score of 1 signified that 1/3 of the A layer contained a pale staining intersublaminar region. The scores for all sections in an LGN were averaged so that each animal was considered a single data point. The mean sublamination score (± S.E.M.) for normal animals was 2.1 ± 0.24 ($n = 6$); an example is shown in Fig. 1A. Control animals ($n = 7$) had a mean score of 1.7 ± 0.22. This score was not significantly different from normal ($P > 0.2$, Student's t-test). The mean score for animals treated with two injections of TTX ($n = 4$) was 2.1 ± 0.4. Treatment with five injections of TTX ($n = 8$) resulted in a sublamination score of 0.94 ± 0.16; an example is shown in Fig. 1B. A summary of scores obtained for all the animals in this study is shown in Fig. 2. Sublamination scores obtained from animals treated with five injections of TTX were significantly less than those obtained from normal ($P < 0.002$, Student's t-test) and control animals ($P < 0.02$). Treatment with two injections of TTX had no effect on sublamination when compared to normal or control animals ($P > 0.3$).

The present results support a role for afferent activity in the refinement of retinogeniculate projections into on/off sublaminae. These findings are consistent with a role for NMDA receptor activation in this process [12]. In addition, they suggest that spontaneous vesicle release at retinogeniculate synapses, which would persist after intraocular TTX application, is insufficient to guide sublaminar refinement. In animals receiving two injections of TTX, sublamination appeared normal. Thus, intermittent activity in retinal projections is sufficient to allow activity-dependent refinement to proceed normally. Interestingly, this neuronal activity is present before eye opening in ferrets (which occurs at around P30), and may represent spontaneous firing of retinal ganglion cells [11,15]. Nearby cells in the retina appear to be coupled to each other electrically by gap junctions [17], and the activity patterns of on-center vs. off-center retinal ganglion cells may differ because the dendrites are differentially stratified in the retina [4]. Spontaneous waves of retinal activity are present before photoreceptor activity, and in the ferret, on-center and off-center retinal ganglion cells participate in distinct waves of activity that are initially indistinguishable but become distinct during the on/off segregation period [26].

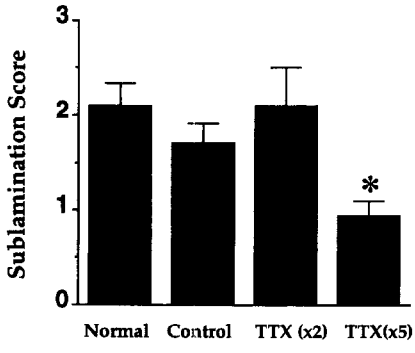

Fig. 2. Histogram summarizing sublamination scores for LGNs in all treatment groups. Error bars denote S.E.M. Treatment with 5 applications of TTX significantly decreased the sublamination score.

While cats lack on/off sublaminae in the LGN, retinal inputs to the LGN are specific for cells with on-center vs. off-center receptive fields [6]. When neonatal kittens are treated with intraocular injections of TTX, LGN cells develop 'on-off' receptive field properties in which a response is seen at the onset of a light stimulus and again when the light stimulus is removed [2]; receptive field properties with this treatment are consistent with non-specific innervation from on, off, X and Y retinal ganglion cells [10]. Intracellular labeling of retinogeniculate axons in cats suggests that activity blockade disrupts the termination patterns of retinal ganglion cells so that X axon arbors are broader and Y axon arbors are narrower and extend into inappropriate eye-specific layers [25]. Thus, changes in axon arbor morphology [23] and synaptic connectivity may underly the disruption of sublamination observed in the present study.

An interesting question is to what extent afferent activity influences both pre- and postsynaptic structures. In the developing cat LGN, blockade of activity with TTX results in an increase in the number of dendritic spines [9], and a similar effect is seen with NMDA receptor blockade in ferrets [18]. While activity may regulate the number of synaptic sites, blockade of activity during retinogeniculate development in tree shrews interferes with the timing but not with the formation of layers formed by cell bodies in the LGN [5]. In the normal ferret, however, Nissl stained material does not reveal clear demarcation of LGN sublaminae at the ages we examined [14]. The formation of patterns in the retinogeniculate pathway may require an interaction between activity-dependent and activity-independent mechanisms. The balance between these mechanisms and the developmental critical periods during which they operate may vary with species and with different

types of patterns. Further studies will be required to understand the nature of interactions between different developmental mechanisms during pattern formation in the nervous system.

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