"N-Methyl-D-aspartate Receptor Antagonist Desegregates Eye-Specific Stripes"

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# *N*-Methyl-D-aspartate receptor antagonist desegregates eye-specific stripes

(optic tectum/ocular dominance columns/correlated activity/aminophosphonovaleric acid/neural maps)

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**ABSTRACT** **The optic tecta of surgically produced three-eyed tadpoles were chronically exposed to the *N*-methyl-D-aspartate (NMDA) receptor antagonist aminophosphonovaleric acid (APV), or to NMDA itself, to assess the influence of NMDA receptor/channels on the eye-specific segregation of retinal ganglion cell (RGC) terminals that occurs whenever two retinas innervate one tectal lobe. Exposure of the tectum to the active isomer of APV produces desegregation of the RGC terminals without blocking electrical activity in the afferents or altering their terminal arbor morphology. Exposure to the inactive isomer of APV causes no perturbation of the normal stripe pattern. APV-induced desegregation is completely reversible within 2 weeks of removal of the APV. In addition, exposure of the optic tectum to NMDA results in stripes with sharper borders and fewer forks and fusions than untreated animals. These results suggest that the NMDA receptor/channel plays a role in eye-specific segregation in the three-eyed tadpole.**

Studies on the establishment of topography in the central visual pathway suggest that retinotopic maps develop by a two-step process, whereby a coarse projection of afferents onto target neurons is followed by a dynamic sorting of relative synaptic positions based on stabilization of coactive afferent terminals. The same cellular mechanisms are believed to be involved in the formation of ocular dominance columns in the visual cortex (1–3), where inputs driven by left and right eyes converge within the same cortical layer. A model system in which to study the fine-tuning of synaptic positions is provided by implanting a supernumerary eye primordium into embryonic *Rana pipiens*. In surgically produced three-eyed tadpoles, retinal ganglion cells (RGCs) from the normal and supernumerary eyes project to the same optic tectum and their terminals segregate into highly stereotyped ocular dominance stripes (4, 5). Despite asymmetric patterns of retinal and tectal cell proliferation (6–8), both retinotopy and eye-specific segregation are maintained throughout the 3–6 months of larval development by a constant shifting of RGC terminals over the tectal surface (1, 9). Consequently, the mechanisms responsible for fine-tuning the retinotectal projection operate continuously throughout larval life, presumably by selectively increasing the lifetimes of coactive connections (10, 11). In the three-eyed frog, disruption of the fine-tuning mechanisms is detectable as a gradual degradation of the stripe pattern (9, 12).

Studies using tetrodotoxin (TTX) to block RGC action potentials have demonstrated that afferent activity is required for the refinement of both the retinotopic projection and eye-specific segregation (13–17). It is thought that retinal inputs confer their neighbor relations to the target neurons by virtue of their highly correlated action potentials (10, 11, 18–21). To understand how correlated inputs may be selectively stabilized, we are attempting to identify the events associated with close temporal activation of multiple inputs to the same postsynaptic cell.

Recent work on synaptic plasticity (22–24) has focused attention on the *N*-methyl-D-aspartate (NMDA) receptor/channel, a subtype of glutamate-sensitive receptor/channel (25–27). The NMDA receptor/channel conducts calcium when the receptor binds transmitter, while the membrane is simultaneously depolarized by other inputs (25, 28, 29) and is therefore uniquely capable of recognizing coincident synaptic activity. In addition, the calcium may trigger biochemical events resulting in synaptic stabilization (30, 31). Since glutamate is a favored candidate neurotransmitter in the RGCs (32, 33), we tested whether activation of the NMDA receptor/channel could play a role in eye-specific segregation in three-eyed tadpoles.

We report that chronic application of aminophosphonovaleric acid (APV), a specific antagonist of the NMDA receptor/channel (34), to the optic tectum of three-eyed tadpoles results in a pronounced but reversible desegregation of RGC terminals without adverse effects on the tectum. APV-induced desegregation of RGC terminals differs from that produced by TTX, because APV has no detectable effect on RGC activity or on RGC terminal arbor size. In addition, chronic application of NMDA itself to the optic tectum appears to produce stripes with sharper borders and fewer forks and fusions. These results suggest that the activation of the NMDA receptor/channel is essential for the fine-tuning of neural maps.

## MATERIALS AND METHODS

DL- or L-APV (1 mM, 0.1 mM, or 0.01 mM), NMDA (0.1 mM), or fluorescein isothiocyanate (0.1 mM) was suspended in the polymer Elvax (35) (a gift of Du Pont) and the solidified polymer was cut on a cryostat into thin slices (≈500 × 500 × 30 μm), which were implanted under the pia over the optic tecta of three-eyed *R. pipiens* tadpoles. After 2.5–5 weeks, the supernumerary retinal projection was labeled with horseradish peroxidase applied to the severed optic nerve. One or 2 days later, the brain underwent reaction with diaminobenzidine tetrachloride in whole mount (9, 36). Subsequently, the tecta were dissected and flattened between coverslips, fixed in 4% paraformaldehyde overnight, dehydrated, and cleared in xylene to produce the flat-mount preparations shown in the figures. Tadpoles were anesthetized by topical application of 0.1% MS222 before all surgical procedures and growth stage was determined according to Taylor and Kollros (37).

Electrophysiological recordings of visually evoked responses were taken with tungsten electrodes from the tectal

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Abbreviations: NMDA, *N*-methyl-D-aspartate; APV, aminophosphonovaleric acid; RGC, retinal ganglion cell; TTX, tetrodotoxin.

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neuropil of tadpoles, which were continuously perfused through the heart with oxygenated Ringer's solution (9).

## RESULTS

The effects of the NMDA receptor/channel antagonist APV and of the agonist itself on the segregation of the RGC terminals in the optic tecta of three-eyed tadpoles are illustrated in Fig. 1. In unoperated or sham-operated tadpoles (Fig. 1*a*), the supernumerary retinal afferents terminate in eye-specific interdigitating bands ≈200 μm wide, oriented along the rostrocaudal axis of the tectum (5) ($n$ = 5). In contrast, the supernumerary retina of animals treated with APV for 4 weeks projects evenly over the tectum ($n$ = 4; Fig. 1*c*). Tecta exposed to APV for shorter periods exhibit an intermediate stage of desegregation (Fig. 1*b*). The dose dependence and timing of the APV-induced desegregation appears to vary with the stages of all nine of the animals showing the effect, such that younger animals, whose tecta grow at a greater rate than older animals, exhibit more complete desegregation at lower APV concentrations or with shorter exposure times than older animals. The APV-induced desegregation was completely reversible within 2 weeks by the removal of the APV-Elvax slice from the brain ($n$ = 4), so that the supernumerary eye projected in a striped pattern indistinguishable from unoperated animals. These data suggest that APV must be present to maintain desegregation. Furthermore, these data indicate that APV is released from the Elvax and is active throughout the exposure times used in these experiments. Exposure of the optic tectum to the inactive isomer L-APV for 4 weeks did not alter the normal striped pattern (Fig. 1*d*; $n$ = 3).

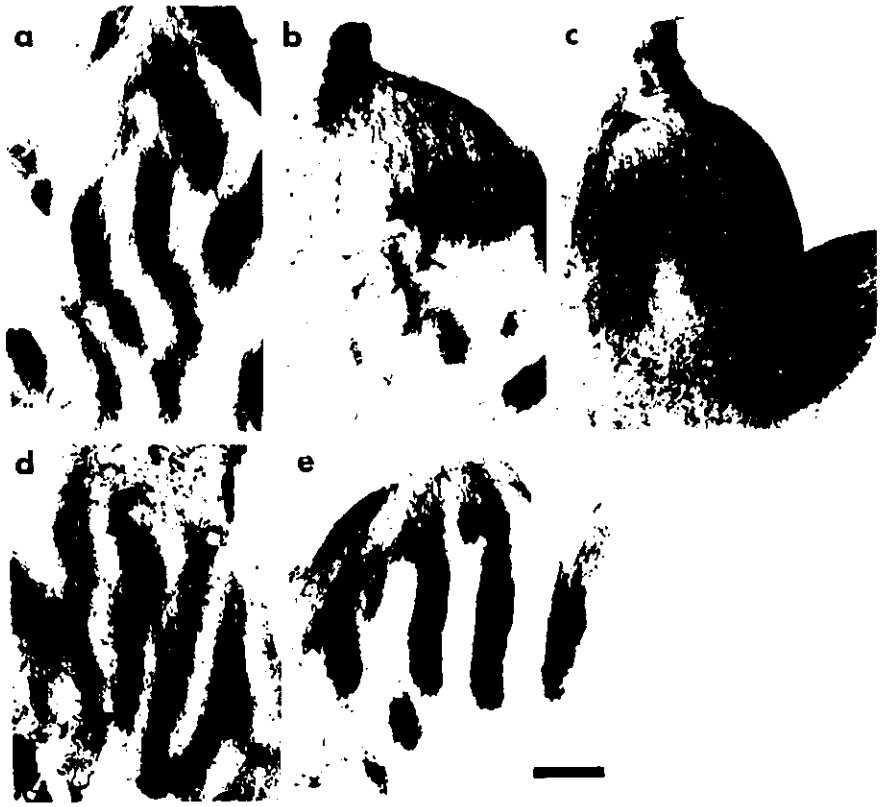


FIG. 1. Effect of the receptor/channel agonist NMDA and of the antagonist DL-APV on segregation of RGC afferents in doubly innervated optic tecta. (*a*) A tectum from a sham-operated animal implanted with fluorescein-conjugated isothiocyanate-Elvax [Taylor and Kollros (T&K) stage XV] (37) shows the stereotypical striped pattern representing the segregation of the supernumerary retinal afferents from the afferents of the normal eye. The segregation of the inputs is not altered by the operation or by the mechanical effect of the Elvax on the tectum. (*b*) A tectum from an animal (T&K stage XIV) treated with DL-APV (0.01 mM) for 2.5 weeks displays an intermediate stage of desegregation. The striped pattern in the rostral tectum has lost its integrity, although periodic variation in horseradish peroxidase staining intensity remains. In the caudal tectum, the terminals are clumped into smaller units called "puffs," which have been previously noted in sparsely innervated tectal lobes (38). (*c*) A tectum from an animal (T&K stage VIII) treated with DL-APV (0.01 mM) for 4 weeks displays complete overlap of the supernumerary and normal retinal projections. Pronounced desegregation was evident in all nine APV-treated animals whose supernumerary optic nerves were labeled with horseradish peroxidase. Four of these animals showed a continuous supernumerary optic nerve projection. (*d*) A tectum from an animal (T&K stage XIV) treated with the inactive isomer of APV (0.1 mM) for 4 weeks displays normal eye-specific segregation. (*e*) A tectum from an animal (T&K stage IX) treated with NMDA (0.1 mM) for 4 weeks. This animal and three others similarly treated show stripes that are straighter and have sharper stripe–interstripe boundaries than control tecta. Rostral is up. (Bar = 200 μm.)



The eye-specific stripes in the tecta of animals chronically treated with NMDA (Fig. 1*e*) differ from the stripes in sham-operated or control tecta in three respects: the stripes in the NMDA-treated tecta are invariably straight and uniform in their periodicity, they have no forks or fusions (38), and the borders of the stripe and interstripe zones are sharper than in controls ($n$ = 4). NMDA treatment appears to increase the capacity of tectal neurons to discriminate between afferents from each eye, resulting in less mixing of uncorrelated inputs in the eye-specific termination zones.

We examined the morphology of RGC terminal arbors and the physiology of visually evoked responses in the optic tecta of APV-treated and untreated animals. The terminal arbors from APV-treated animals have the same tangential area as the arbors from untreated animals of the same age (Fig. 2). Furthermore, extracellular recordings taken from the optic tectum during the presentation of visual stimuli reveal that RGC afferent activity in APV-treated animals is indistinguishable from that in normal animals (Fig. 3).

APV-induced desegregation is not due to toxic effects of the drug on either the tectum or the retinal afferents. As mentioned above, the APV-induced desegregation was completely reversible after removal of the APV-Elvax slice. Histological examination of sectioned tecta treated with APV revealed no obvious disruption in cell number, lamination, or neuropil thickness. In addition, we estimated cell densities in layer 6 by counting cells in a 625-$\mu m^2$ × 10-μm volume under ×400 magnification (38). Cell densities from untreated and APV-treated tecta were not significantly different (15.7 ± 1.6 cells per 6250 $\mu m^3$ and 15.4 ± 0.5 cells per 6250 $\mu m^3$,

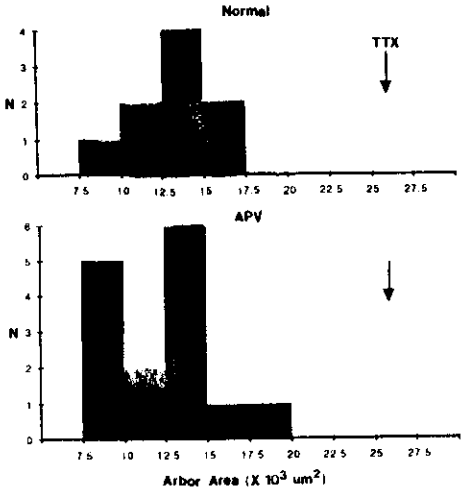


FIG. 2. The number of RGC terminals (N) plotted against their tangential area for untreated animals (mean area ± SD = 13.3 × $10^3$ ± 2.5 × $10^3$ $\mu m^2$; $n$ = 9; *Upper*) and for DL-APV-treated tadpoles (mean area ± SD = 12.2 × $10^3$ ± 2.8 × $10^3$ $\mu m^2$; $n$ = 15; *Lower*). The areas occupied by arbors of the two groups are not significantly different (two-tailed $t$ test), whereas the mean area occupied by arbors from TTX-treated animals (12) is 25.3 × $10^3$ $\mu m^2$ (arrow), about twice the size of the controls.

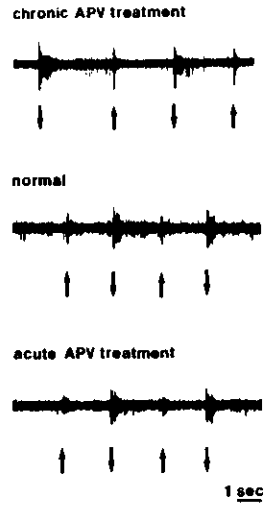


FIG. 3. Visually evoked responses recorded from the optic te of tadpoles chronically treated with DL-APV ($n$ = 3) (*Top*), untrea ($n$ = 5) (*Middle*), and acutely treated by topical application of A (0.1 mM in Ringer's solution) to the exposed optic tectum ($n$ = (*Bottom*). Lights were turned on (upward arrows) and off (downw arrows) as indicated under each trace. In animals treated chronic with APV, the entire dorsal surface of the tectum had been expo to APV-Elvax (1 mM) for 5–6 weeks prior to the recording sessi To control for the possibility that any effect of chronically appl APV on spike invasion into the afferent terminals was washed out the perfusion system before the recordings were taken, we app APV solution directly to the exposed optic tectum (i.e., acut treated) and tested for the presence of afferent activity every 10 for 1 hr.

respectively; $n$ = 3 for each group). In short, no signs of damage or death were observed in treated animals.

## DISCUSSION

The results demonstrate desegregation of ocular dominan stripes by the specific NMDA receptor/channel antagon APV. We suggest that activation of the NMDA recept channel allows tectal neurons to recognize and maint coactive afferent synapses, while inappropriate converg inputs are lost. Because ganglion cells that are reti neighbors are known to be coactive (18–21), whereas t activity pattern in non-neighboring RGCs is relatively unc related, coactive neighboring RGCs whose arbors conver on the same population of tectal neurons would activ NMDA receptor/channels as their postsynaptic potenti summate. Normal activation of the NMDA receptor/chan would initiate a process of synapse stabilization and there preserve the neighbor relations in the target tissue during formation of the retinotopic map. Exogenous NMDA wo desensitize the NMDA receptors so that a higher degree afferent coactivity is required to stabilize synapses, result in stripes with sharper borders. Blocking the NM receptor/channels would prevent recognition of affer coactivity and eliminate selective synapse stabilizati thereby producing desegregation in the face of normal p terns of presynaptic activity. Singer *et al.* (22) have repor that chronic infusion of APV into the striate cortex of kitt blocks the physiologically determined ocular dominance s

normally seen in response to monocular deprivation. These results are consistent with the role we propose for the NMDA receptor/channel in the structural stabilization of coactive visual synapses.

Chronic application of TTX to the optic nerves of three-eyed tadpoles results in the desegregation of the striped pattern in the tectum that is similar to the effect of chronic APV treatment and occurs over roughly the same time period (12). However, TTX-induced desegregation and APV-induced desegregation differ in at least three respects: TTX blocks visually evoked activity in the retinal afferents, the RGC terminal arbors of TTX-treated animals occupy 2–3 times as much area as arbors from untreated animals, and growth cones are more prevalent on the RGC terminals of TTX-treated animals (12). There are three possible interpretations for TTX-induced desegregation. First, TTX-induced desegregation might be due to sprouting of RGC terminals, since decreased action potential activity facilitates sprouting in cultured *Helisoma* neurons (39). Alternatively, TTX, by decreasing the activation of tectal cells, may cause desegregation through a mechanism similar to that proposed for the neuromuscular junction (40). Namely, inactive target neurons would actively, but nonselectively, recruit inputs by releasing a sprouting factor. Finally, TTX-induced desegregation may be attributed to a disruption of the process of synaptic stabilization. We believe that the APV-induced desegregation lends support to the third interpretation of the TTX-induced desegregation. Our results suggest that desegregation and sprouting are governed by different mechanisms and that TTX-induced desegregation results from the indirect block of postsynaptic NMDA receptor/channels.

This study suggests a general mechanism for the development and maintenance of neural maps in the vertebrate central nervous system based on a scheme originally proposed by Hebb (10, 11). The model we present closely parallels the proposed cellular mechanisms underlying long term potentiation (LTP) in the CA1 region of the hippocampus, where a particular spatiotemporal pattern of pre- and postsynaptic activity produces a long-lasting increase in synaptic efficacy through the activation of postsynaptic NMDA receptor/channels (23, 24, 41–45). The desegregation of eye-specific stripes in APV-treated animals provides anatomical evidence for a role of the NMDA receptor/channel in determining the relative positions of synaptic inputs in central nervous system projections. Our data, in the context of the extensive work on LTP, suggest that a single mechanism has properties that would allow it to control structural refinement in the developing nervous system and plasticity in the mature brain.

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