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Restoration of the Plasticity of Binocular Maps by NMDA After the Critical Period in *Xenopus*

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Visual input during a critical period of development plays a major role in the establishment of orderly connections in the developing visual system. In *Xenopus laevis*, the matching of visual maps from the two eyes to the optic tectum depends on binocular visual input during the critical period, which extends from late tadpole to early juvenile stages. Alterations in eye position, which produce a mismatch of the tectal maps, normally evoke a compensatory adjustment in the map of the ipsilateral eye only during the critical period. However, continuous application of the glutamate receptor agonist *N*-methyl-D-aspartate (NMDA) after the normal end of the critical period restores this ability to realign the visual map.

VISUAL EXPERIENCE CAN HAVE A major impact on the developing axonal connections of the visual system. In particular, factors such as imbalance or misalignment of input from the two eyes alter the axonal connections that underlie binocular maps. The period during which a set of connections is modifiable is referred to as the critical period for that projection (*1*). The cellular events that normally lead to loss of plasticity during maturation are still unknown. During the critical period, application of drugs that interfere with synaptic function can interfere with plasticity. For example, blockage of NMDA-type glutamate receptors prevents visually evoked reorganization of ocular dominance columns in kitten visual cortex (*2*) and of binocular maps in the tectum of juvenile *Xenopus laevis* (*3*). These results have led us to consider whether normal loss of plasticity reflects a decrease in NMDA receptor function and to test whether application of NMDA can reverse the normal loss of plasticity in *Xenopus* tectum.

In *Xenopus*, each tectal lobe receives binocular input. The projection of the contralateral eye is relayed directly by way of the optic nerve, whereas the projection of the ipsilateral eye is relayed indirectly, by way of the opposite tectal lobe and the nucleus isthmi (*4*) (Fig. 1). The two projections form topographic maps of the visual field in the tectum that are in register with each other. The map for the ipsilateral eye comes into alignment with the map for the contralateral eye by active adjustment of axonal connections during development (*5*), when the ipsilateral projection displays dramatic plasticity (*6*). For example, if one eye is rotated by 90° in a midlarval tadpole, while the other eye remains in its normal orientation, the maps of the two eyes initially are misaligned by 90°. However, over the next 2 to 3 months, the ipsilateral map on each tectal lobe becomes reoriented to match the rotated contralateral map (*7*). This realignment normally occurs only during the critical period of development, which ends at about 3 months after metamorphosis. As the frog ages, the ipsilateral map loses the capacity to reorient in response to eye rotation (*8*).

What processes bring these two maps into register? Visually elicited neuronal activity appears to be crucial, since alignment of the ipsilateral map to match the contralateral map requires binocular visual input during development (*5*). One mechanism proposed for activity-dependent refinement of retinotectal maps in frogs and fish (*9*) involves selective stabilization of those retinotectal synapses located on tectal cells receiving input from other retinotectal axons that fire in a correlated pattern. Such correlation is most likely to occur for axons with overlapping receptive fields. The stabilization process seems to be triggered by activation of the NMDA-type glutamate receptor (*10, 11*). Sufficient activity would depolarize tectal cell dendrites by non–NMDA-type glutamate receptors, and the depolarization would expel Mg^{2+} ions from the NMDA receptor channel, allowing influx of Na^+ and Ca^{2+} (*12*). The Ca^{2+} could induce some transient change in the dendrite, allowing it to stabilize recently active retinotectal synapses. We propose a comparable mechanism for activity-dependent matching of the ipsilateral (isthmotectal) and contralateral (retinotectal) maps in *Xenopus*. When isthmotectal and retinotectal axons with corresponding receptive fields converge on a given tectal cell, the NMDA-dependent mechanism would stabilize the isthmotectal synapses (Fig. 2). Consistent with this proposed role for NMDA receptors, we have shown that in *Xenopus*, blocking the NMDA receptor during the critical period of development prevents the ipsilateral map from

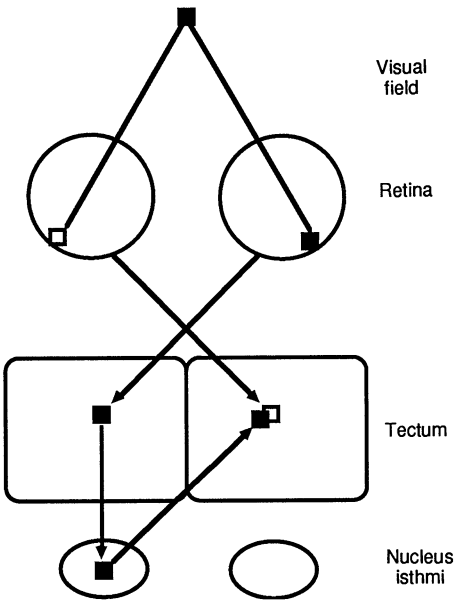

Fig. 1. Neural connections from the eyes to the optic tectum. The right lobe of the tectum receives input from the left eye directly by way of the retinotectal projection and from the right eye indirectly by way of the left nucleus isthmi.

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coming into alignment with the contralateral map (*3*).

The reduction in plasticity that normally occurs after the critical period could result from a decrease in the number or effectiveness of NMDA receptors. If this were the case, plasticity might be restored artificially by boosting the activity of NMDA receptors. To test this possibility, we applied NMDA continuously to the tectum in 15 juvenile *Xenopus laevis* frogs. At 8 months after metamorphosis, well past the normal end of the critical period, the left eye was rotated by 90° clockwise. On the same day, we began to apply NMDA by a 40- to 50-μm-thick slice of slow release Elvax polymer, impregnated with 0.1 or 0.4 mM NMDA, inserted beneath the pia of the right tectal lobe (*3*). In four control animals, a vehicle-containing Elvax implant was applied, and in four others no implant was inserted. After 12 to 24 weeks, the contralateral and ipsilateral maps were determined by standard electrophysiological techniques (*3, 13*).

We measured receptive field locations from electrode penetrations at comparable tectal positions (Fig. 3D) in five representative frogs. The ipsilateral and contralateral units recorded at each tectal site are in register in a normal frog with no eye rotation (Fig. 3A). The positions of the ipsilateral and contralateral units are misaligned in frogs in which one eye was rotated at 8 months after metamorphosis, with no drugs applied (Fig. 3, B and C). The contralateral positions are rotated by 90° relative to normal, reflecting the 90° rotation of the left eye, whereas most ipsilateral positions are normally oriented, reflecting the normal orientation of the right eye. In contrast, after NMDA treatment of late-juvenile frogs, eye rotation produces a different result (Fig. 3, E and F): the ipsilateral units have shifted such that their fields are in register with the contralateral positions. These maps display the plasticity normally seen only at younger ages.

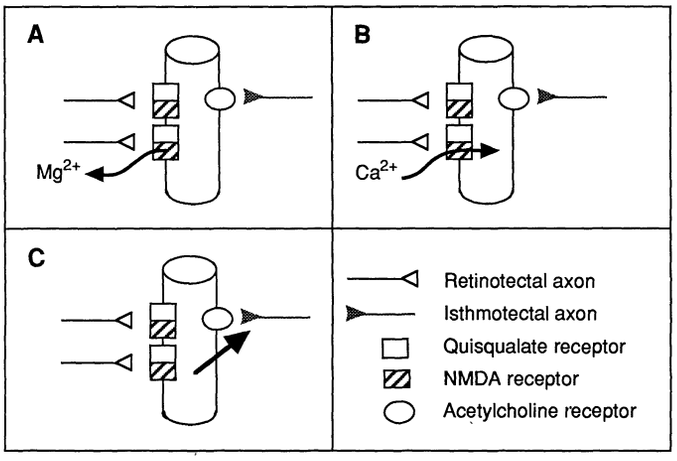

Fig. 2. Hypothetical events underlying activity-dependent stabilization of isthmotectal synapses. (**A**) Release of glutamate by retinotectal axons produces depolarization of tectal cell dendrites via quisqualate receptors. (**B**) The depolarization expels Mg^{2+} ions from NMDA receptors (A), which then respond to binding of glutamate by allowing influx of cations such as Na^+ and Ca^{2+}. (**C**) Calcium could trigger a transient release of a trophic substance (arrow) that stabilizes an isthmotectal cell that had been primed by recent firing.

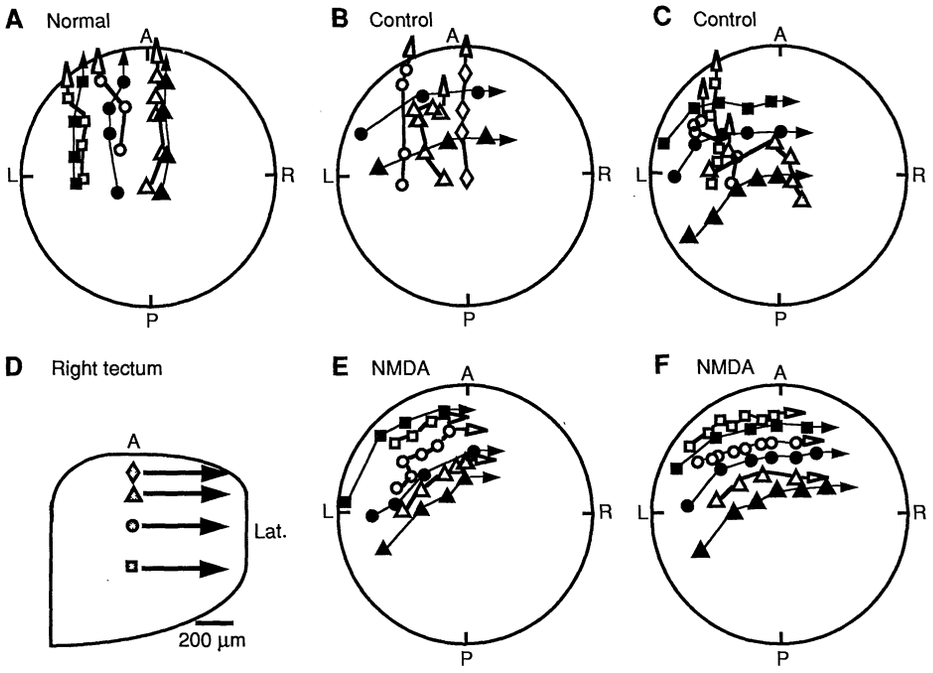

Fig. 3. Visual field maps recorded as in (*3*). (**A**) Normal, 12 months after metamorphosis. (**B** and **C**) Left eye rotated at 8 months post-metamorphosis. Visual field maps were recorded 15 (B) and 12 (C) weeks later. (**D**) Schematic dorsal view of tectum showing locations of the electrode penetrations. (**E** and **F**) Left eye rotated and NMDA (0.4 mM)-impregnated Elvax inserted at 8 months after metamorphosis; recorded 13 (E) and 16 (F) weeks later. In some cases, we recorded ipsilateral fields from tectal positions where we had not recorded contralateral units before cutting the contralateral optic nerve. Filled symbols, contralateral eye; open symbols, ipsilateral eye; A, anterior; P, posterior; L, left; R, right; Lat., lateral.

To compare the degree of alignment of maps from control and NMDA-treated frogs, we measured the difference in the azimuths (left-right coordinates) of the pairs of units recorded at each tectal location (Fig. 4) (*14*). We averaged the values for all pairs of points in each map to obtain a score for each animal. Frogs with eye rotations performed at 8 months after metamorphosis without NMDA treatment displayed significantly more misalignment than normals (Mann-Whitney two-tailed U test, $n_1 = 5$, $n_2 = 8$, $U = 0$, $U' = 40$, $P < 0.01$), whereas the frogs treated with NMDA after the critical period showed a normal degree of alignment compared with normals ($n_1 = 5$, $n_2 = 15$, $U = 16$, $U' = 59$, $P > 0.05$) and had values significantly different from the untreated frogs ($n_1 = 8$), $n_2 = 15$, $U = 8$, $U' = 112$, $P < 0.01$). No significant difference existed between the two doses of NMDA used ($n_1 = 8$, $n_2 = 7$, $U = 27$, $U' = 29$, $P > 0.10$). The scores for the representative maps in Fig. 3, A through C and E through F, are indicated by the corresponding letters in Fig. 4.

There was some sign of reorganization in the maps from the control animals subject to late eye rotation. Parts of the ipsilateral maps were substantially disorganized after eye rotation. These data imply that the ipsilateral axons in control frogs retained some ability to shift to new positions in response to the misalignment of the eyes, but that they were incapable of using binocular activity cues to come into proper alignment with the contralateral fields. Moreover, in control animals, it became progressively more difficult to record ipsilateral units beyond 16 weeks after the eye rotation; those units that were found had receptive fields clustered near the center of rota-

tion of the receptive field of the left eye, the region where the least mismatch had been induced by the rotation. Such a pattern could result from a loss or dispersion of branches of isthmotectal axons, particularly those representing the periphery of the field, where the induced mismatch between the ipsilateral and contralateral maps was greatest. In contrast, no such loss of peripheral ipsilateral fields occurred in the NMDA-treated frogs. The absence of fields with the greatest potential disparity in the maps from untreated tecta reduced the apparent contrast between those maps and the maps from the NMDA-treated tecta.

One interpretation of these results is that plasticity is normally limited by a reduction in the number of NMDA receptors during development (*15*). Such a decrease could bring the NMDA receptor–mediated Ca^{2+} fluxes below a threshold that is necessary to trigger synaptic plasticity. Chronic NMDA could restore plasticity by prolonging the time during which NMDA receptors remain open and thus permitting sufficient influx of Ca^{2+} to trigger the stabilizing mechanism. A complication with this model is the possibility that chronic treatment with NMDA could desensitize the response of the tectum to NMDA (*16*). In order to explain how chronic NMDA treatment promotes activity-dependent sharpening of ocular dominance bands in surgically produced three-eyed frogs, Cline and Constantine-Paton (*11*) suggest that this desensitization makes the criteria for activating the receptors more stringent, with only the most closely corresponding activity patterns leading to stabilization. Thus, only the best matches would be retained. Such an effect would lead to the prediction that the tectum might be less responsive to visual input as well, but our studies of tectal output activity after continuous NMDA treatment indicate that the tectum is more rather than less responsive to visual input than normal (*17*). It is not known whether this change is a result of alterations in NMDA receptors. It is unlikely that the ability of NMDA to restore plasticity in *Xenopus* after the critical period is somehow mediated by neurotoxicity, as the doses of NMDA used in our experiments (*18*) and in studies of surgically produced three-eyed *Rana pipiens* produced no significant toxic effects on tectal cells (*11*).

It is not known whether there are NMDA receptors on the terminals of isthmotectal axons or whether activation of such receptors could directly promote sprouting. Our studies of chronic NMDA treatment during the critical period (*3*) did not reveal any differences in mapping from age-matched controls, but anatomical studies will be required to establish whether such presynaptic

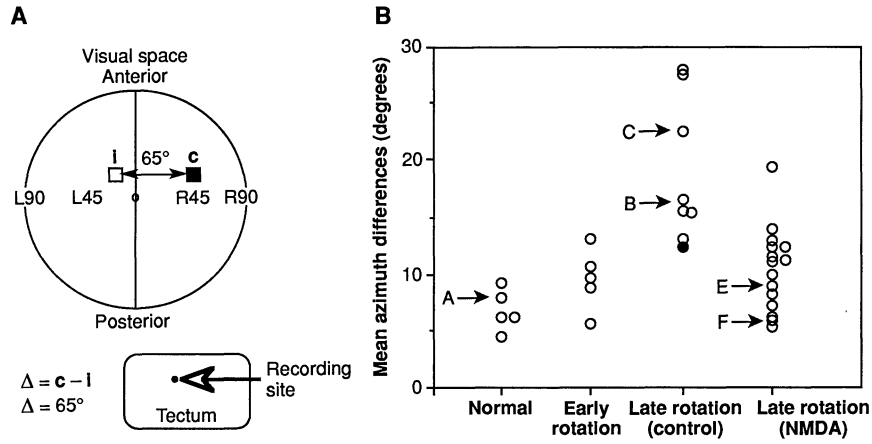

Fig. 4. Quantitative analysis of the effects of NMDA on plasticity. (**A**) For each tectal site, the azimuth (left-right) distance between the ipsilateral and contralateral receptive fields recorded at that site is computed. Perfect alignment yields a value of 0. For each frog, the azimuth distances for all such pairs were averaged to give a single value. (**B**) Each circle represents the mean azimuth distance recorded in an individual frog; ●, 24 weeks after eye rotation, a frog in which ipsilateral units were found only for positions near the axis of eye rotation. Early rotations were performed before metamorphosis. Late rotations were performed at 8 months after metamorphosis. The age range of the normal *Xenopus* was from 2 months to 1 year after metamorphosis. Because no statistically significant difference existed between the groups for the two doses of NMDA, the data from both dosage groups are combined. Arrows indicate data from corresponding maps in Fig. 3.

effects do occur. There is evidence in the retinotectal system, in which NMDA can affect activity-dependent refinement of maps, that NMDA treatment per se does not alter axonal branching patterns (*11*).

One question raised by our data is why the isthmotectal axons alter their connections so little in the absence of exogenous NMDA. There is only minor disruption of the normal pattern of isthmotectal connections and a gradual deterioration of the map in our control frogs despite the dramatic mismatch of activity patterns that comes about as a consequence of the eye rotation. Such changes could be the manifestation of a gradual spreading of the terminal arbors of the isthmotectal axons, as the mismatched visual inputs fail to provide the necessary stabilization to maintain the integrity of the map. This idea implies that even after the critical period in frogs, activity cues still can exert some low-level effect, which normally serves to keep the binocular maps in register during the period of life when the eyes continue to grow, and the retinotectal map to shift, at very slow rates (*5*).

Our model implies that isthmotectal axons do not directly trigger the opening of NMDA receptor channels, but that the synapses of those axons must be close enough to such channels to respond to their activation. Anatomical studies indicate that the isthmotectal and retinotectal axons terminate in close proximity to each other in two laminae in the superficial neuropil of the tectum (*19*). The two sets of axons do not make morphologically identifiable synapses with each other but both contact tectal cell dendrites.

Whatever the mechanism, our results indicate that in frogs, the capacity of mature axons to reorganize in response to sensory input can be restored or boosted to that normally observed during the critical period just after metamorphosis.

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cut behind the eye; and the ipsilateral map was recorded.
14. We did not include data from those sites at which we recorded only contralateral input or from those sites that received input from the monocular region of the contralateral eye's visual field. No statistically significant difference was observed between the control tecta with Elvax implants and those without. The data are therefore presented together. Data for azimuth alone rather than angular separation were analyzed because of slight tipping of the head in some frogs when the optic nerve was cut after mapping the contralateral projection.
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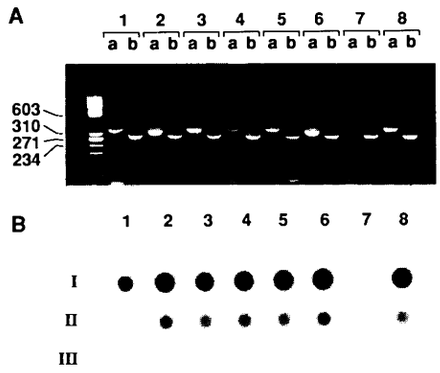

Fig. 1. (**A**) Amplified products from eight uveal melanoma tissues are shown after 35 cycles of PCR with 5′-sense primer of $V_\alpha 7$ and 3′-primer of C_α as shown in Table 1 (lanes a) and primers for melanotransferrin (L-MEL 1; 5′-TAC CTG GTG GAG AGC GGC CGC CTC-3′, R-MEL 2; 5′-AGC GTC TTC CCA TCA GTG T-3′) (lanes b). The PCR conditions were the same as in Table 2. The size of amplified products obtained with melanotransferrin primers was 286 bp. The leftmost lane shows the marker sizes. Lane number indicates the case numbers of melanoma samples. (**B**) Dot blot hybridization of amplified cDNA from eight melanoma tissues. Row I (10 μl) and row II (1 μl) of each amplified product obtained with $V_\alpha 7$ and C_α primers as described in the legend to (A) were hybridized with [γ-^{32}P]-labeled $V_\alpha 7$ oligonucleotide probe (*17*). As a negative control, the PCR product of melanotransferrin primers was also blotted (row III, 10 μl per sample). Case numbers were the same in Fig. 1 and Table 2. After each sample of PCR-amplified product was denatured with 0.4 M NaOH for 10 min at room temperature, they were applied to nitrocellulose paper (GeneScreen Plus, Du Pont Biotechnology Systems). The paper was fixed with ultraviolet (UV) light for 10 min, then prehybridized in 5× SSPE (0.18 M NaCl, 10 mM phosphate (pH 7.4), and 1 mM EDTA), 5× Denhardt's solution, 0.1% SDS containing salmon sperm DNA at 42°C for 3 hours. Thereafter samples were hybridized for 12 to 16 hours with 5×10^6 to 8×10^6 cpm of ^{32}P-labeled $V_\alpha 7$ oligonucleotide probe at 42°C. After hybridization, blots were washed twice in 1× SSPE and 0.1% SDS at room temperature for 10 min and placed under x-ray film (X-O mat AR; Kodak) for 30 min to 2 hours to develop (*18*).

Predominant Expression of T Cell Receptor $V_\alpha 7$ in Tumor-Infiltrating Lymphocytes of Uveal Melanoma

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Expression of T cell receptor (TCR) V_α genes in tumor-infiltrating lymphocytes (TILs) within intraocular melanoma was studied. Primers for 18 different human TCR V_α families were used to analyze TCR V_α-C_α gene rearrangements in TIL in these melanomas obtained at surgery. A limited number of TCR V_α genes were expressed and rearranged in these tumors, and TILs expressing $V_\alpha 7$ were found in seven of eight of these uveal melanomas. TCR gene usage is also restricted in experimental autoimmune disease, in T cells within organs like skin and other epithelial tissues, and in the brain of patients with multiple sclerosis (MS). The restricted usage of TCR genes in TIL may indicate that a specific antigen in these melanomas is targeted.

IDENTIFICATION OF THE SPECIFICITY of T cells involved in the local immune response to malignant tumors is an important step in the development of cancer immunotherapy. The use of expanded populations of TILs for therapy appears promising in animal models of solid tumor and in human clinical trials of melanoma (*1*), although the TCR genes expressed in TILs are not yet known. Recent evidence from studies in autoimmunity (*2, 3*) and allograft rejection (*4*) indicates that effector cells may utilize a very limited range of TCR genes. We asked whether there was restricted heterogeneity of TCR expression in TILs.

We chose to study melanoma of the uveal tract, which includes the pigmented portion of the eye, the iris, ciliary body, and choroid, because circumstantial evidence suggests that this tumor elicits an immune response that may protect the host (*5, 6*). In humans with uveal melanoma and in murine models of ocular melanoma, antitumor immunity to melanoma antigens is inducible (*7*). Melanoma of the uveal tract are often fatal, with nearly half of the patients dying within 10 to 15 years of enucleation (*7*). The primary lesions of uveal melanoma, like those of its cutaneous counterpart, may remain localized for long periods, metastatic disease may ensue only decades later, and spontaneous remissions occur (*6*).

To determine the extent of TCR gene usage by TILs, we examined the diversity of V_α genes in transcripts of rearranged TCR α-chain genes in eight uveal melanoma specimens obtained at surgery (*8*). Various methods could have been used to address this question, including immunohistochemical staining for TCRs, Northern blotting of mRNA from tissues, or extended in vitro culture of TILs (*9*). Unfortunately, a wide panel of monoclonal antibodies (MAbs) specific for TCR V_α regions in humans is unavailable. Also mRNA or DNA from TIL in surgical specimens is often scarce. Long-term culture of TILs in vitro may bias analysis of the TCR repertoire. Therefore, we decided to exploit gene amplification by polymerase chain reaction (PCR). The PCR method allows enzymatic amplification of a target DNA sequence. By selection of the appropriate oligonucleotides, the method is sensitive enough to detect a DNA sequence from one TIL in a population of 10^5 cells within a solid tumor mass (*10*).

To analyze the usage of V_α genes, we analyzed the cDNA reverse transcribed from mRNA isolated from uveal melanoma specimens. Eighteen different V_α-specific oligo-

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