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# Newly Learned Auditory Responses Mediated by NMDA Receptors in the Owl Inferior Colliculus

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Alignment of auditory and visual receptive fields in the optic tectum of the barn owl (*Tyto alba*) is maintained through experience-dependent modification of auditory responses in the external nucleus of the inferior colliculus (ICX), which provides auditory input to the tectum. Newly learned tectal auditory responses, induced by altered visual experience, were found to be pharmacologically distinct from normal responses expressed at the same tectal sites. *N*-methyl-D-aspartate (NMDA) receptor antagonists administered systemically or applied locally in the ICX reduced learned responses more than normal responses. This differential blockade was not observed with non-NMDA or broad-spectrum antagonists. Thus, NMDA receptors preferentially mediate the expression of novel neuronal responses induced by experience during development.

Experience-dependent modification of neuronal responses tailors the function of neural circuits based on the sensory experience of the individual. Pharmacological studies of this process (*1*) have implicated the NMDA subtype of excitatory amino acid (EAA) receptor in the induction of experience-dependent synaptic modification. However, interpretation of these experiments is difficult because the specific effects of NMDA receptor blockade are usually confounded with nonspecific effects of blocking postsynaptic activity (*2*). We have used a different approach in a system in which normal and newly learned responses can be recorded simultaneously at single sites. Here we show that newly functional circuitry, once it has been induced by experience-dependent processes, is pharmacologically specialized: Transmission through this circuitry is preferentially mediated by NMDA receptors, relative to transmission through original circuitry (*3*).

Barn owls localize sounds using interaural timing difference (ITD) as a cue for sound source azimuth. In the ICX, where the owl's map of auditory space is synthesized, neurons are tuned to specific ITD values and are organized into a map of ITD, and hence of azimuthal space. The auditory space map is relayed topographically to the optic tectum, where it is aligned with the tectal map of visual space so that tectal neurons are tuned to the value of ITD produced by sounds at the locations of their visual receptive fields (VRFs) (*4*). This alignment is dynamically maintained by experience-dependent plasticity and can be altered systematically if owls are raised wearing prismatic spectacles that optically displace the visual field in azimuth (*5*, *6*). During prism-rearing, tectal neurons develop novel responses to sounds with ITDs that correspond to the location of their optically displaced VRFs (schematized in Fig. 1A). At many tectal sites, these novel responses, which are to ITDs that are systematically displaced from the normal ITD range (*6*), first appear while responses to ITDs in the normal range continue to be expressed, creating a "transition state" ITD tuning curve (*7*) (Fig. 1A, middle panel). Transition state tuning curves are often abnormally broad and sometimes double-peaked. They are defined here as those ITD tuning curves that include both responses to ITD values that are normally appropriate for that tectal site, termed "normal responses," and responses to ITD values corresponding to the prismatically displaced VRF, termed "learned responses" (*8*). Over subsequent weeks, normal responses are eliminated to produce a narrow ITD tuning curve centered on the learned ITD value (Fig. 1A, bottom panel).

The alteration of tectal ITD tuning can be accounted for by experience-dependent plasticity that occurs at the level of the ICX (*6*, *7*). In the study reported here, we compared the pharmacology of ICX circuits mediating normal and newly learned responses in prism-reared owls. We did this by applying EAA receptor antagonists either systemically or locally into the ICX while recording responses at tectal sites displaying transition state ITD tuning. We focused on NMDA and non-NMDA subtypes of EAA receptors, because auditory transmission in the ICX of normal owls is known to be mediated through these receptors (*9*). ITD tuning was monitored in the optic tectum rather than in the ICX, because tectal VRFs allow unambiguous determination of normal ITD tuning for any given site (*6*, *8*).

Systemic injection of the anesthetic and NMDA receptor antagonist ketamine HCl (*10*) at 10 to 15 mg per kilogram of body weight, a dose known to selectively antagonize NMDA receptors in the ICX (*11*),

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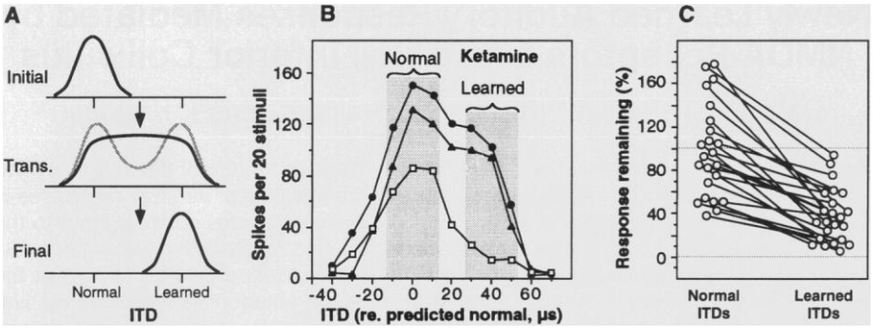


**Fig. 1.** Differential suppression of learned responses by systemically injected ketamine. (**A**) Development of transition state ITD tuning (Trans.) at a single tectal site. Initially after prism attachment, ITD tuning is centered at a value appropriate for the VRF (top). Newly learned responses (middle) emerge over several weeks at ITD values corresponding to the prismatically displaced VRF, creating broadly tuned (black) or double-peaked (gray) tuning curves. Responses to the normal range of ITDs later disappear (bottom). (**B**) ITD tuning at a tectal site displaying transition state tuning, recorded 0 to 20 min before (circles), 0 to 20 min after (squares), and 68 to 87 min after (triangles) injection of ketamine HCl (10 mg/kg). Values of ITD are indicated relative (re.) to the normal ITD value for that site, calculated from the VRF (*8*). Positive ITD values indicate the direction of prism-induced shift in ITD tuning. Gray regions denote normal and learned responses (*8*). (**C**) The effect of ketamine at all sites tested ($n = 23$). For each site, connected circles denote mean blockade of normal and learned responses in the 20 min after injection, relative to control levels. Data points above 100% indicate responses that increased after ketamine injection; such increases were interpreted to mean that inhibitory circuitry, driven by NMDA receptor-mediated activity, normally suppressed responses at those sites.

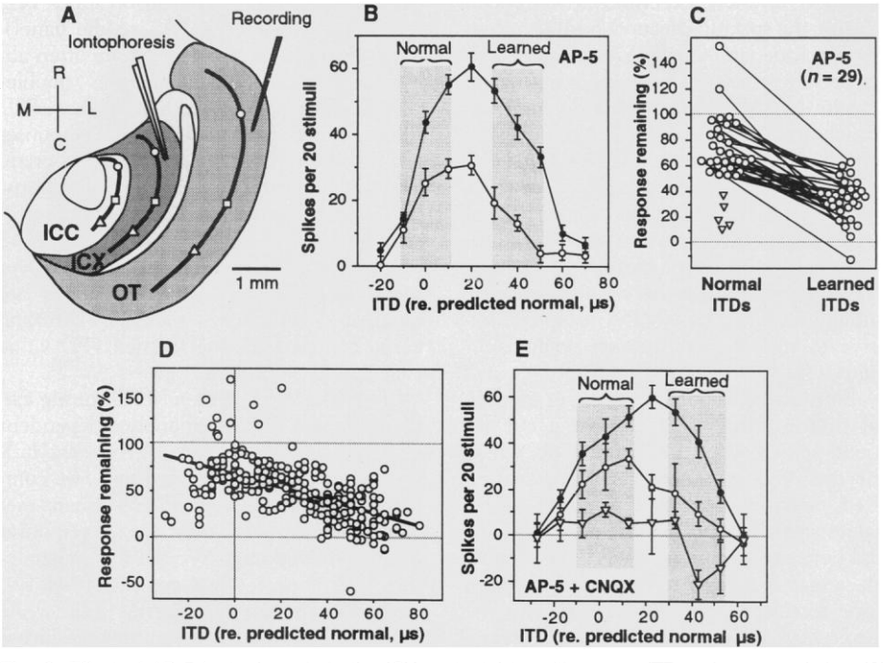


**Fig. 2.** Effect of AP-5 iontophoresis in the ICX on tectal transition state ITD tuning; re., relative. (**A**) Location of iontophoresis and recording electrodes on a schematic horizontal section through the optic tectum (OT) and external (ICX) and central (ICC) nuclei of the inferior colliculus. Locations of units normally tuned to 0 (circles), 50 (squares), and 100 μs (triangles) ITD are shown on the axis of varying ITD tuning in each nucleus (thick lines). (**B**) Effect of AP-5 iontophoresis (40 nA) in the ICX on transition state ITD tuning at a tectal site. Each curve represents mean tuning over several interleaved control and recovery (solid circles) or AP-5 (open circles) periods (*15*). Bars indicate SEM of 6 to 12 curves of 20 repetitions each. (**C**) The effect of AP-5 on normal and learned responses at all sites tested. AP-5 reduced learned responses more than normal responses (Wilcoxon signed rank test, $P < 0.0001$). Triangles indicate the effect of combined AP-5 + CNQX on normal responses at five sites. (**D**) Relation between stimulus ITD relative to predicted normal ITD and response suppression produced by AP-5, for all sites. Analysis was restricted to ITDs for which control responses were ≥15% of the maximum observed at each site. Line indicates least-squares regression ($r^2 = 0.32$, slope = −0.007, $P = 0.0001$). (**E**) Effect of combined AP-5 + CNQX (triangles) at ejection currents producing maximal tectal response suppression (80 nA each). Open circles, 40 nA AP-5 alone.

preferentially reduced learned responses relative to normal responses at tectal sites exhibiting transition state ITD tuning (*12*). A representative site is shown in Fig. 1B. The effect of systemic ketamine was measured at 23 tectal sites displaying transition state ITD tuning in five prism-reared owls (Fig. 1C). For each site, the magnitude of response blockade was calculated separately for normal and learned responses (Fig. 1B, gray areas). Ketamine preferentially reduced learned responses at all sites (*13*). On average, ketamine reduced learned responses by 62% while reducing normal responses by only 2% from control levels. In contrast, Valium (0.5 to 1 mg/kg), a drug with no known action at NMDA receptors, reduced both normal and learned responses equally [learned responses were reduced by 43% and normal responses were reduced by 41%, respectively, relative to control ($n = 4$)].

To test the hypothesis that the effect of systemic ketamine was due to antagonism of NMDA receptors in the ICX, the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP-5) was iontophoresed into the ICX while recording from tectal sites that displayed transition state ITD tuning. AP-5 was iontophoresed at an ICX site that was topographically matched (*14*) to the tectal recording site (Fig. 2A) and was applied with ejection currents (15 to 40 nA) known to selectively antagonize NMDA receptors relative to non-NMDA receptors (*9*). AP-5 was applied during one or more 20- to 30-min periods interleaved with control (recovery) periods (*15*). Consistent with the known role of NMDA receptors in mediating ICX auditory responses (*9*), the overall level of response was depressed during periods of AP-5 iontophoresis. In addition, AP-5, like ketamine, preferentially reduced learned responses relative to normal responses (Fig. 2B). The effect of AP-5 iontophoresis in the ICX was measured at 29 tectal sites in 12 prism-reared owls. AP-5 reduced learned responses more than normal responses at every site (Fig. 2C). In a second analysis, response blockade was calculated separately for each ITD tested. A significant correlation existed between the ITD of a stimulus, relative to the predicted normal ITD value, and the degree to which AP-5 blocked its response (Fig. 2D). There was no correlation between the response decrement caused by AP-5 and the original magnitude of the control response (*16*). This preferential blockade of learned responses by AP-5 was apparent even though ITD tuning at the iontophoresis site always matched either the transition state tuning or the normal tuning for the tectal site (*14*). To rule out the possibility that the AP-5–resistant normal responses might be mediated by non-EAAergic synapses or by synapses outside of the ICX, larger amounts (80 nA) of AP-5 were

applied in combination with the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; also 80 nA) at some sites (*17*). Combined AP-5 and CNQX application greatly reduced normal responses, relative to the reduction achieved by NMDA receptor–specific amounts of AP-5 alone (Fig. 2E; triangles in Fig. 2C).

To exclude the possibility that newly learned responses might be preferentially susceptible to blockade by any EAA receptor antagonist (as might be the case if circuits mediating them involved more synapses than did circuits mediating normal responses), CNQX or the broad-spectrum EAA receptor antagonist kynurenic acid (KYN) were applied using the same protocol as for AP-5 (Fig. 3). Unlike AP-5, both these drugs reduced normal responses as much or more than learned responses (Fig. 3, A, C, and D). Across all sites tested, KYN ($n$ = 10) blocked responses to all ITDs equally (Fig. 3B), whereas CNQX ($n$ = 9) showed a slight preferential blockade of responses to ITDs near the normal value (Fig. 3E). We observed that KYN and CNQX reduced overall tectal responses less, on average, than did AP-5, despite larger iontophoretic currents. This is consistent with biochemical properties of KYN and CNQX (*18*) that predict smaller regions of effective concentration for these drugs than for AP-5. To determine if the relatively small reduction of overall responses observed with KYN and CNQX was responsible for the apparent difference in the effects of these drugs relative to AP-5, we identified six sites at which AP-5 and either KYN or CNQX reduced overall responses by approximately the same amount (Fig. 3F). At each of these sites, AP-5 reduced learned responses preferentially whereas KYN or CNQX either reduced responses equally or reduced normal responses preferentially (*19*).

We conclude that NMDA receptors in the ICX contribute differentially to the expression of learned responses, at least during the period when learning is taking place. The result that ketamine and AP-5, but not KYN or CNQX, greatly reduce learned responses is consistent with a model in which these responses are mediated primarily by NMDA receptors. In contrast, normal responses, which are reduced by all antagonists, are likely to be mediated by both NMDA and non-NMDA receptors (*20*). This model is supported by the observation that newly learned tectal responses have a longer latency and time course than do normal responses (*7*). Experiments in other developmental systems have focused on the role of NMDA receptors in the induction of novel responses (*1*), as opposed to their expression, and thus our results suggest a new role for NMDA receptors in synaptic modification during development. Precedents for NMDA receptors mediating the expression of synaptic modification are found in metabotropic glutamate receptor–induced potentiation in the dentate gyrus, anoxic long-term potentiation (LTP), and the kindling model of epilepsy (*21*).

In normal owls, the ratio of NMDA to non-NMDA receptor contribution to ICX auditory responses is constant across all ITDs to which a given neuron responds (*9*). Thus, the present results indicate that prism-rearing causes the appearance of two pharmacologically distinct populations of synapses mediating transmission in separate functional circuits in the ICX (*22*). These synapses may differ in the relative numbers of NMDA and non-NMDA receptors they contain or in other factors that alter the relative amounts or efficacies of NMDA and non-NMDA receptor currents (*23*). For instance, it is possible that synapses mediating learned responses may have pure NMDA receptor pharmacology, like synapses observed recently in the hippocampus (*24*). How pharmacologically distinct synaptic populations develop during prism-rearing is unknown; possibilities include the formation or functional activation of synapses enriched in NMDA receptors to mediate learned responses, or the existence of an NMDA receptor–specific form of LTP.

The current results also suggest an alternative interpretation of experiments in which chronic application of NMDA receptor antagonists prevents experience-dependent synaptic modification during development (*1*). Such experiments have been used to argue that NMDA receptor currents trigger synaptic enhancement in these systems, as they do in LTP (*25*). The present results suggest that NMDA receptor antagonists may also prevent plasticity through a specific blockade of newly learned postsynaptic responses. Blockade of the earliest learned responses would prevent the concurrent pre- and postsynaptic activation required for continued enhancement of these responses, and thus learned responses would fail to develop. It remains to be determined whether NMDA receptors

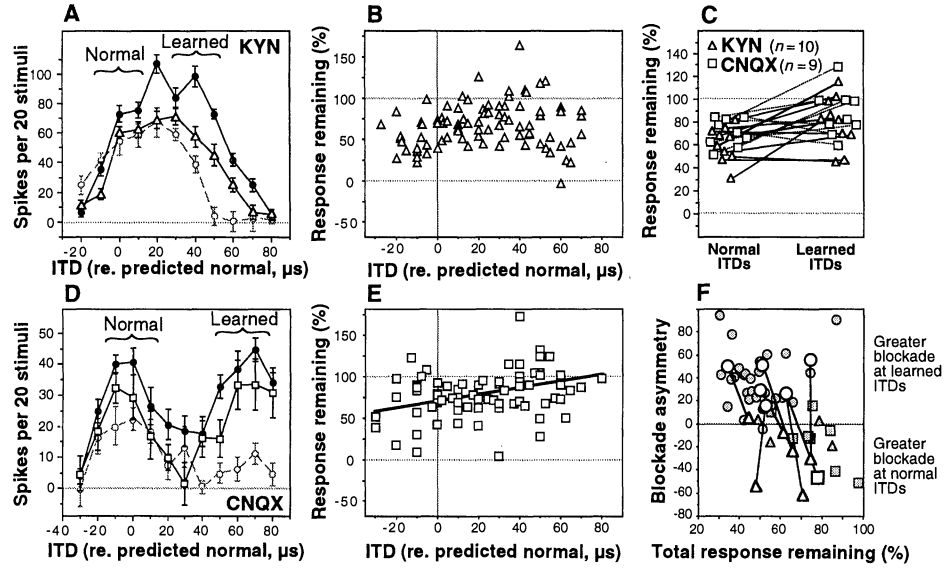


**Fig. 3.** Effects of KYN and CNQX iontophoresis in the ICX on tectal ITD tuning; re., relative. (**A** and **D**) Representative effects of KYN (triangles) and CNQX (squares) at two tectal sites. The effect of AP-5 at the same sites is shown for comparison (open circles, dashed lines). Solid circles, control and recovery periods. The typical effect of KYN was to block both normal and learned responses equally. Though CNQX preferentially blocked normal responses at some sites, the magnitude of this effect was small, and thus the representative site in (D) shows an equal blockade of normal and learned responses. Responses during CNQX application were significantly reduced relative to control responses at this site ($P < 0.005$) (*26*). (**B** and **E**) Relation between stimulus ITD relative to predicted normal ITD and response decrement produced by KYN (triangles) or CNQX (squares), for all sites. KYN: $r^2 = 0.024$, slope = 0.002, $P = 0.14$. CNQX: $r^2 = 0.117$, slope = 0.004, $P = 0.002$ (regression line shown). (**C**) Summary of the effects of KYN and CNQX on normal and learned responses. Neither KYN nor CNQX blocked learned responses more than normal responses (Wilcoxon signed rank test, one-tailed, each $P > 0.25$). (**F**) Relation between overall response blockade and relative blockade of normal versus learned responses for all sites tested with AP-5 (circles), KYN (triangles), or CNQX (squares). Abscissa, response blockade calculated across all ITDs sampled. Ordinate, difference in percentage of control spikes blocked at normal and learned ITDs. Connected symbols represent data from individual sites at which the overall response blockade produced by AP-5 matched that produced by KYN or CNQX within 15%.

play this role in the expression of learned responses in other models of developmental plasticity.

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8. For each tectal site, we measured the VRF with prisms off and calculated the normal ITD tuning peak in microseconds as (2.5 × VRF azimuth in degrees) −2.7, from (*6*). The range of normal responses was defined as responses to ITDs within ±15 μs of this value. The range of learned responses was defined as responses to ITDs 40 ± 15 μs displaced from the normal ITD value or, for double-peaked tuning curves, as responses to ITDs within ±15 μs of the abnormal response peak.
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11. Ketamine injections reduced responses of ICX neurons to iontophoresed NMDA but not to the non-NMDA receptor agonist quisqualate. Responses to iontophoresed NMDA returned to control levels ≈50 min after ketamine injection. Learned responses at transition state sites in prism-reared owls showed recovery that followed a similar time course.
12. Multiunit recordings were made in the superficial layers of the optic tecta of owls raised wearing 23° laterally displacing prismatic spectacles. Prism-rearing, dichotic stimuli, and recording procedures were as in (*6*), and iontophoresis electrodes and solutions were as in (*9*). Owls were unanesthetized during data collection except for ketamine injections used to assess tuning changes.
13. The effects of ketamine, AP-5, CNQX, and KYN on transition state ITD tuning curves were independent of the direction of prismatic displacement (right or left), the location of the normal VRF within frontal space, and the laterality of the recording site.
14. The ICX projects topographically to the tectum in normal owls [E. I. Knudsen and P. F. Knudsen, *J. Comp. Neurol.* **218**, 187 (1983)], and the topography is unchanged in prism-reared owls [D. E. Feldman and E. I. Knudsen, unpublished results]. In prism-reared owls, transition state ITD tuning develops in the lateral portion of the ICX while the medial portion retains largely normal tuning. Therefore, an ICX site was considered topographically matched to a tectal site if it was located in the medial ICX and was tuned to an ITD matched within 15 μs to the normal ITD of the tectal site, or if it was located in the lateral ICX and had ITD tuning that matched the tectal transition state tuning.
15. ITD tuning curves in Figs. 2 and 3 are means ± SEM of all curves collected during all control and recovery periods or during all drug application periods. On average, responses declined only 3% between the first and last control periods. Drug ejection was verified by observation of local blockade of auditory responses at each ICX iontophoresis site. Drug iontophoresis significantly reduced tectal auditory responses at all sites reported in this study (*26*).
16. D. E. Feldman, M. S. Brainard, E. I. Knudsen, data not shown.
17. The 80-nA current level for AP-5 and CNQX was chosen because it produced maximal tectal response blockade in both normal owls (79 ± 10% reduction of control responses, $n$ = 17 sites) and prism-reared owls (82 ± 11% reduction, $n$ = 5 transition state sites).
18. These properties are the hydrophobicity of CNQX and the relatively high concentration of KYN required for receptor blockade [G. L. Collingridge and R. A. J. Lester, *Pharmacol. Rev.* **40**, 143 (1989)].
19. Positional differences between ICX synapses mediating learned and normal responses cannot explain these results. If synapses mediating learned responses were located farther from the iontophoresis site than were pharmacologically identical synapses mediating normal responses, then all drugs, including AP-5, should block normal responses preferentially. Conversely, if synapses mediating learned responses were located nearer the iontophoresis site, then CNQX and KYN should preferentially block these responses, but they do not.
20. At many sites (Fig. 3D), CNQX blocked normal and learned responses equally. This may reflect a direct blockade of non-NMDA receptors together with an indirect reduction of NMDA receptor currents due to the voltage dependence of this receptor class [R. A. Nicoll, R. C. Malenka, J. A. Kauer, *Physiol. Rev.* **70**, 513 (1990)].
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