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NMDA Antagonists in the Superior Colliculus Prevent Developmental Plasticity But Not Visual Transmission or Map Compression

L. HUANG and S. L. PALLAS

Graduate Program in Neurobiology and Behavior, Department of Biology, Georgia State University, Atlanta, Georgia 30303

Abstract

Partial ablation of the superior colliculus (SC) at birth in hamsters compresses the retinocollicular map, increasing the amount of visual field represented at each SC location. Receptive field sizes of single SC neurons are maintained, however, preserving receptive field properties in the prelesion condition. The mechanism that allows single SC neurons to restrict the number of convergent retinal inputs and thus compensate for induced brain damage is unknown. In this study, we examined the role of *N*-methyl-D-aspartate (NMDA) receptors in controlling retinocollicular convergence. We found that chronic 2-amino-5-phosphonovaleric acid (APV) blockade of NMDA receptors from birth in normal hamsters resulted in enlarged single-unit receptive fields in SC neurons from normal maps and further enlargement in lesioned animals with compressed maps. The effect was linearly related to lesion size. These results suggest that NMDA receptors are necessary to control afferent/target convergence in the normal SC and to compensate for excess retinal afferents in lesioned animals. Despite the alteration in receptive field size in the APV-treated animals, a complete visual map was present in both normal and lesioned hamsters. Visual responsiveness in the treated SC was normal; thus the loss of compensatory plasticity was not due to reduced visual responsiveness. Our results argue that NMDA receptors are essential for map refinement, construction of receptive fields, and compensation for damage but not overall map compression. The results are consistent with a role for the NMDA receptor as a coincidence detector with a threshold, providing visual neurons with the ability to calculate the amount of visual space represented by competing retinal inputs through the absolute amount of coincidence in their firing patterns. This mechanism of population matching is likely to be of general importance during nervous system development.

INTRODUCTION

Early in mammalian brain development, neurons are over-produced and extend collaterals widely. At a later stage, reductive processes such as programmed cell death and collateral elimination partially correct this exuberance (see Blaschke et al. 1998; Hamburger 1992; O'Leary and Koester 1993 for review). Formation of the developing mammalian retinocollicular projection involves convergence of multiple retinal ganglion cells onto each

collicular target cell. Retinal ganglion cells follow graded molecular cues to form a roughly retinotopic visual map in the superficial layers (O'Leary and Wilkinson 1999 for review) and then undergo a period of axon branch refinement (Simon and O'Leary 1992), resulting in approximately 10 ganglion cells providing input to each SC neuron in hamsters (Finlay and Pallas 1989; Stone and Pinto 1993). At the population level, the extent of this convergence determines the grain of the retinotopic map and thus perceptual acuity. At the single neuron level, summation of the receptive field properties of convergent inputs results in derivative properties in individual collicular cells, such as receptive fields that are larger than those of the ganglion cells (Hubel and Wiesel 1962). It is not understood what processes direct the establishment of different afferent/target convergence ratios in different structures and even within topographic maps either at the population or single-cell level. As afferents converge on target neurons, information about individual loci within the sensory epithelium and separation of parallel processing streams is lost. Conversely, increasing the number of separate sampling points increases perceptual acuity (Lee et al. 1992; Tootle and Friedlander 1989). Thus the nervous system must have a means to control how many and which inputs converge on each target cell despite the initial overproduction. Although there have been many studies on the role of both activity-dependent and -independent processes in the development and plasticity of topographic maps, few have addressed the consequences of mapping rules for single-target cells.

In hamsters the retinocollicular projection forms largely after birth, and the convergence and map refinement processes can be challenged by inducing a form of developmental plasticity called map compression. When the caudal half of the superior colliculus (SC) is ablated at birth, the retinal axons form a compressed but complete retinotopic map on the rostral SC fragment (Finlay et al. 1979). Under these conditions, although multi-unit receptive field sizes increase as expected in representing a larger area of visual field on a smaller surface, single-unit receptive field sizes are maintained at normal values (Pallas and Finlay 1989). This paradoxical result occurs as a result of compensatory changes in retinal axon arbor morphology (Pallas and Finlay 1991; Xiong et al. 1994), resulting in a conservation of the amount of visual space represented by each SC neuron and thus a preservation of function. How this population matching is achieved on a mechanistic level in either normal or compressed maps, or even whether the process depends on neuronal activity, is not understood and is the subject of this study.

It is reasonable to suspect that *N*-methyl-D-aspartate (NMDA) receptors play a critical role in the convergence control process in the SC. NMDA receptors are thought to mediate changes in synaptic efficacy in the developing nervous system, as they do in adult hippocampal long-term potentiation (LTP), by detecting coincident activity in converging inputs (see Cline 1998; Larkman and Jack 1995 for review; Constantine-Paton and Cline 1998). The retinocollicular map refinement process in developing rats can be disrupted by NMDA receptor blockade (Simon et al. 1992), suggesting that it depends on Hebbian-type activity-dependent stabilization. To demonstrate a specific role for NMDA receptors in activity-dependent stabilization, however, it is necessary to demonstrate that they are not required for retinocollicular transmission. In contrast to mammalian sensory neocortex (Fox and Daw 1993; Kasamatsu et al. 1998; Miller et al. 1989), NMDA receptors are only minimally involved in normal synaptic transmission in the frog tectum (Cline et al. 1987;

Udin et al. 1992) and its homologue, the mammalian SC (Binns and Salt 1998a,b; Okada 1993; Roberts et al. 1991; Schnupp et al. 1995). In addition, the component of the postsynaptic current due to the NMDA receptor at birth is small and approximately the same in the developing SC as in the hippocampus (Binns and Salt 1998a,b; Clark et al. 1994; Collingridge et al. 1983; Müller et al. 1988). Thus one component of this study was to determine whether visual responses were normal after NMDA receptor blockade in hamster SC and thus whether we could test the coincidence detection role of NMDA receptors independent of any loss in activity.

To test the hypothesis that NMDA receptors are necessary for the conservation of receptive field properties in compressed maps, we recorded responses to visual stimuli from single SC units in hamsters raised with chronic application of NMDA receptor antagonists to the SC throughout postnatal development and analyzed the effect of the receptor blockade on map topography, map refinement, and receptive field size in compressed and normal retinocollicular maps. We found that blockade of NMDA receptors left visual responses and overall map topography intact in both normal and compressed maps but prevented conservation of receptive field size and map refinement. These results provide support for a critical and specific role of NMDA receptors in mammalian developmental plasticity and reveal a mechanism for population matching that is likely to be of general importance in brain development.

METHODS

Animals

Forty-five Syrian hamsters (*Mesocricetus auratus*) were used in this study. Experimental animals were bred in the Georgia State University animal facility from breeding stock purchased from Charles River. Normal animals were either purchased as adults or bred in the colony. All the procedures used met or exceeded standards of accepted care developed by the Institutional Animal Care and Use Committee, the American Physiological Society, the National Institutes of Health, and the Society for Neuroscience.

Experimental design

To determine the role of NMDA receptor activity in map formation, refinement, maintenance, and matching of afferent/target populations during normal development, NMDA receptors in the SC were exposed throughout development to the biologically active form of the NMDA receptor antagonist amino-5-phosphonovalerate (D-APV). The inactive isomer (L-APV) was used as a control. After rearing the hamsters to adulthood under these conditions, maps and receptive fields were assessed electrophysiologically. To determine the role of NMDA receptor activity in developmental plasticity, NMDA receptor blockade was combined with surgically-induced map compression. Five groups of animals were used. The normal (N) group ($n = 8$) received neither surgical nor drug treatment. The partial tectum (PT) group ($n = 6$) had partial ablations of the caudal superior colliculus on the day of birth. The D-APV group ($n = 16$) had a D-APV-impregnated piece of Elvax (see following section) implanted over the superior colliculus at birth, and the L-APV group ($n = 5$) was implanted with L-APV-impregnated Elvax as a control for any nonspecific effects of placing the Elvax

on the SC. In the PT/D-APV group ($n = 10$), Elvax was first inserted over the part of SC to be spared; then the caudal SC was heat cauterized. The Elvax was inserted under the pia in each case to reduce the likelihood that forebrain was exposed to the drug.

Control experiments to assess receptor pharmacology were done using iontophoresis of drugs coupled with single-unit recordings, in some cases combined with visual stimulation. The drugs used included: glutamate (Glu), NMDA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), D-APV, and 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX).

Elvax preparation

The APV-impregnated Elvax was generously donated by Adam Smith (University of Oxford, UK) and was prepared according to published procedures (Silberstein and Daniel 1982; Smith et al. 1995). Briefly, an aqueous solution of L- or D-APV (Tocris Neuramin, Langford, UK) in Elvax 40P (DuPont, UK) with 5% aqueous Fast Green FCF (Allied Biochemical, Morristown, NJ) was mixed to give a final concentration of 10 mM APV. A small amount (1:100,000) of tritiated APV was incorporated in the Elvax to provide a measure of drug release rate. Drugs delivered in this way have an initial burst of release and then a steady release for at least 60 days (Cline and Constantine-Paton 1989; Schnupp et al. 1995; Smith et al. 1995). To avoid exposing the SC to the initial burst of APV release from the Elvax, 100- μ m-thick sheets of Elvax were preincubated for 48 h in phosphate-buffered saline (PBS; pH 7.4; 0.5 ml) at 37°C prior to implantation. The amount of APV released during this 48-h period was calculated from scintillation counts (Fig. 1) (mean = 706.4 ± 45.45 pmol/mm² per 48 h). We incubated five sheets of Elvax another 2 days without implanting them to determine how quickly the release rate falls off (mean = 133.2 ± 17.38 pmol/mm² per 48 h). After this, the release was more gradual and a low rate of release (mean = 39.6 ± 4.52 pmol/mm² per 48 h) persisted for at least 10 mo. One animal used at 12 mo of age had a negligible amount of remaining APV release from the Elvax.

Neonatal surgery

Neonatal surgery was performed within 12 h of birth. The hamster pups were deeply anesthetized with 4% isoflurane in oxygen and maintained on 1–2% isoflurane. For partial SC ablation (PT group), the skin over the midbrain was incised and the SC visualized through the thin skull (the cortex has not grown over the SC at birth). The superficial layers of the caudal portion of the right SC were ablated by heat cauterization, and the wound was closed with 6-0 Prolene. For the D- or L-APV implantation groups, an incision was made through the skull and the pia mater at the boundary between superior and inferior colliculi, and a sheet of Elvax was cut to fit and slipped under the pia and over the SC. Following the surgery, the pups were returned to the dam for rearing. The growing cortex pushes the Elvax implant rearward. To determine how long the Elvax remained on the SC, we examined the brains of eight D-APV/Elvax-implanted neonates at intervals. We found that the Elvax remained covering the superior colliculus until P25. This is sufficiently late in SC development that the map would have been formed and refined (Finlay et al. 1982; Hofer et al. 1994; Sengelaub et al. 1986; Warton and McCart 1989). All subsequent manipulations were done at adulthood except where specifically mentioned.

Adult surgery

Prior to preparation for physiological recording, animals were anesthetized with urethan (0.7 g/ml; 0.03 ml/kg body weight in 3–4 ip aliquots spaced at 20-min intervals). Respiration rate and withdrawal reflexes were monitored to ensure that a surgical plane of anesthesia was maintained, with supplemental doses of urethan given as needed. The pupils were dilated with a 10% ophthalmic atropine solution. After a craniotomy was performed, the sagittal sinus was ligated and cut. The visual cortex was aspirated bilaterally to eliminate influences from corticocollicular projections (Rhoades and Chalupa 1976, 1978), and to expose the SC. For D-APV, L-APV, and PT/D-APV groups, the position of the Elvax piece was noted prior to removing it for analysis. The brain was kept covered with sterile saline for protection. The animal was then put in a stereotaxic device with the head fixed at an angle of 30° up from horizontal to orient the surface of the SC horizontally. The conjunctiva at the nasal corner of the left eye was anchored by a suture to the stereotaxic frame to stabilize eye position. The eye was covered with a fitted plano contact lens for protection during the recording session.

Electrophysiological recording

For extracellular multi-unit and single-unit recording, parylene-coated tungsten microelectrodes (Frederick Haer, Bowdoinham, ME) with a tip diameter of 1–2 μm were used. Using a penlight as a search stimulus, electrode penetrations were made perpendicular to the surface of the SC to locate visually responsive areas in the retino-recipient superficial gray layer (SGS) (Pallas and Finlay 1989). Single units were isolated by spike height and shape from multi-unit activity using a window discriminator. Data were acquired by CED 1401 hardware (Cambridge Electronic Design, Cambridge, UK) and processed on- and off-line by Spike2 software (donated by Kaare Christian, Rockefeller University).

In lesioned animals, recording sessions commenced with rapid multi-unit mapping of the rostrocaudal extent of the remaining SC to determine the extent of the lesion, whether the visual map was compressed, and whether the entire visual field was represented. This electrophysiological estimate of lesion size was confirmed histologically after the experiment was complete. To address the role of NMDA receptors in compression of the retinotopic map, we mapped the locations of multi-unit receptive fields across the rostrocaudal extent of the SC, assessing the regularity and completeness of the maps in each experimental group. Recording sites were approximately 100 μm apart in rostral SC (nasal visual field) and 200 μm apart in caudal SC (peripheral visual field).

Once the mapping was completed, single units from nasocentral visual field were carefully isolated and recorded in detail. This region of the SC exhibits regular compression of the retinal representation in response to partial SC ablation (Finlay et al. 1979). In each penetration, only the first well-isolated unit encountered was used, and all units chosen were within 100 μm of the SC surface. The uniformity of receptive field size is maximized by choosing this recording region and depth (Fortin et al. 1999; Tiao and Blakemore 1976). The “classical” receptive field of each isolated unit was manually demarcated on four sides with a penlight or by using computer-generated stimuli consisting of small white spots on a dark screen. The receptive-field diameter of each unit was defined as the distance from the nasal to the temporal boundary of the plotted field (the axis of map compression). In some cases,

the detailed shape of the receptive field was also delineated by multiple bidirectional sweeps of a spot 0.5° in diameter at 1.0° intervals throughout the receptive field. Receptive field size distributions between groups were compared with a one-way ANOVA using a post hoc Tukey test for the multiple pairwise comparisons. There were no statistical differences in receptive field sizes determined manually or with computer-generated stimuli, and thus these data are combined in the following text except where noted. Each lesioned animal has a different lesion size and represents an independent observation, and thus in the text the means of all measurements within each animal are given rather than all cells within a group of animals. For comparison, the data are also presented in tabular form using both units and animals as individual observations. Variability in the data are represented by the standard error of the mean.

Visual stimulation

A frosted hemisphere (for manual plotting of fields) or a 14-in computer display monitor was placed in front of the hamster's left eye at a distance of 30 cm (manual plotting) or 40 cm (computer plotting), and the data were converted into degrees of subtended angle. The optic disk was localized with a reversible ophthalmoscope and plotted onto the center of the stimulus hemisphere (for manual plotting) or at a fixed location with respect to the computer screen. The position of the optic disk was replotted periodically to ensure that the eye position was stable.

A Sargent Pepper graphics board (Number Nine, Cambridge, MA) was used in conjunction with "STIM" software (Kaare Christian, Rockefeller University) to generate simple visual stimuli consisting of single, smoothly moving light spots that could be varied in diameter, direction, and velocity. A minimum intertrial interval of = s was used to prevent adaptation. Both manual and computer-generated stimulus paradigms were used to plot the receptive fields. In either case, bidirectionally swept 0.5° spots at interstimulus distances of 1.0° were used to determine the receptive-field edges along the nasotemporal axis. It is important to note that we could not place the computer monitor in an exactly tangential way for units above and below the horizontal meridian, resulting in artificial compression of the dorsoventral extent of the receptive field plot for outlying units. This does not bias our conclusions because we used only nasotemporal receptive-field diameter in the statistical comparisons.

Iontophoresis

For iontophoresis of glutamate receptor agonists and antagonists, multi-barrel glass electrodes (FHC, Bowdoinham, ME) broken to a tip diameter of $5\text{--}8\text{ }\mu\text{m}$ ($1\text{--}2\text{ }\mu\text{m}$ per barrel) were used. Extracellular single-unit activity was recorded through a 3 M NaCl-filled barrel. The remaining electrode barrels were used for iontophoretic drug application and were filled with the AMPA receptor antagonist CNQX (1 mM, pH 8.4), the NMDA receptor antagonist D-APV (50 mM, pH 8.0), or the agonists glutamate (1 M, pH 7.5–8.0), AMPA (10 mM, pH 8.3), or NMDA (0.1 M, pH 7.5–8.0). Saline (165 mM NaCl) was used as a balance barrel or as a control. Neurochemicals were obtained from RBI/Sigma (Natick, MA). Drugs were delivered to effect and were ejected with negative current from an iontophoresis device (Cygnus Technologies, Delaware Water Gap, PA), using the following ejection parameters:

glutamate, 10 s, 50–150 nA; AMPA, 10–20 s, 40–120 nA; NMDA, 10–20 s, 85–150 nA; CNQX, 20–30 s, 10–60 nA; and D-APV, 20–30 s, 2–10 nA. Visual stimuli presented during iontophoresis consisted of sweeping spots of light optimized for each neuron. Action potentials from individual neurons were isolated with a waveform discriminator and related temporally to the drug application using CED hardware and software. Statistical comparison of iontophoretic data was done using nonparametric methods (Mann-Whitney rank-sum test).

Histology

At the termination of each recording session, under deep anesthesia, animals were perfused through the heart with PBS (0.1 M, pH 7.4) followed by 10% buffered formalin. Brains were removed, postfixed for 10–12 h, and stored in PBS containing 30% sucrose for cryo-preservation. They were then sectioned frozen in the coronal plane at 50 μ m and mounted for Nissl staining with cresylecht violet (Chroma Gesellschaft, Münster, Germany). Neurolucida and Neuromorph software (MicroBrightfield, Colchester, VT) were used to analyze the volume of the superficial layers of the SC in both normal and lesioned hamsters.

RESULTS

Map topography is unaffected by NMDA receptor antagonists

The topography of the retinotopic maps in normal and lesioned animals was determined by multi-unit mapping. Maps were complete in their extent in all animals, meaning the retinocollicular maps in the PT (partial ablation of SC) and PT/D-APV groups were compressed (Fig. 2). The amount of map compression was linearly related to lesion size. Furthermore there were no localized inversions or irregularities observed, suggesting that the cues that normally guide the formation of the initial retinotopic map, regardless of the size of the SC, are not affected by the drug treatment.

NMDA receptor blockade increases receptive field size of single units in normal maps

We measured the receptive-field (RF) sizes of single units in the superficial layer of unlesioned superior colliculi using extra-cellular recording techniques and either manual or computer-aided plotting (Fig. 3). With hand-plotted fields, the upper, lower, nasal, and temporal edges were defined (Fig. 3A). With computer-aided plotting (Fig. 3B), a small light spot was swept up and down across the screen, delineating the RFs on the nasotemporal axis along which the lesions were made. We found that fields were fairly symmetrical and that they increased in size in the periphery. The fields were clearly larger in the D-APV animals and were further increased in size in the PT/D-APV animals (see following text). Although the RFs differed in size, they were generally similar in shape when plotted by hand (Fig. 3A). The edges of the largest fields were somewhat less well defined in the APV-treated animals, as might be expected in an unrefined projection, and were more difficult to plot.

The level of responsiveness of individual SC neurons to visual stimulation might be expected to vary in the APV-treated animals. Although our RF plotting methods were designed to be independent of response level, we did measure the responsiveness of a representative sample of neurons within each experimental group. We found that the mean

level of response to visual stimulation was not significantly different between groups [normal 36 ± 7.1 (SE) spikes per visual stimulus; PT 22 ± 9.5 ; L-APV 27 ± 5.4 ; D-APV 25 ± 2.6 ; PT/D-APV 29 ± 4.0 ; $P > 0.1$ in all pairwise comparisons; Fig. 4]. We also failed to observe any drug-induced changes in spontaneous activity, although we did not analyze this factor quantitatively. These cells rarely exhibit spontaneous activity (Tiao and Blakemore 1976), and we avoided recording from the few cells encountered that did, given the difficulty in plotting the RFs of such units. Within these limitations, we did not observe increases in spontaneous activity or any treatment-related change in the overall responsiveness of the neurons to visual stimulation.

The mean RF diameter of the units in normal animals was $9.2 \pm 0.40^\circ$ ($n = 8$ animals, 40 units; Table 1, Fig. 5). If NMDA receptors are necessary for limiting convergence of ganglion cells onto SC neurons during refinement of normal maps, then we would expect to see larger than normal RFs in the D-APV animals. We found that blockade of NMDA receptors (D-APV group) caused a 50% overall increase in RF size, yielding a mean of $15.1 \pm 0.22^\circ$ ($n = 16$ animals, 59 units; Fig. 5, *A* and *B*). The population as a whole shifted to larger field sizes as can be seen in the scatter plot of the data (Fig. 5*C*). The distributions of RF sizes in the normal and D-APV groups were compared using a one-way ANOVA, and the values were found to be significantly different ($P < 0.001$).

An additional group using implants of L-APV in Elvax was included to control for any nonspecific effects of the Elvax polymer, independent of the drug treatment (L-APV is the biologically inactive isomer of APV). There was no significant difference ($P = 0.06$) between RF size distributions from the normal and L-APV groups (L-APV mean $10.4 \pm 0.18^\circ$, $n = 5$ animals, 35 units) or between the PT and L-APV groups ($P = 0.9$). As mentioned in the preceding text, a previous study showed that RF properties are conserved after partial neonatal ablation of superior colliculi (Pallas and Finlay 1989), so we expected RF size to be normal in the PT group. In contrast, the RF sizes in the L-APV and PT groups were significantly different from the D-APV group ($P < 0.001$). The L-APV data argues that any artifacts that may have resulted from the Elvax implantation procedure were not statistically significant.

NMDA receptor blockade further increases RF size in compressed maps

In a previous study, it was demonstrated that RF diameter is maintained despite partial SC ablation (Pallas and Finlay 1989), suggesting that the number of afferents converging on each target cell is regulated at single SC cells. We found that mean RF diameter in animals with partial ablation of the SC (PT) but without APV treatment was $10.1 \pm 0.17^\circ$ ($n = 5$ animals, 35 units; Fig. 5, *A* and *B*). The distribution of RF diameters in the normal and PT groups was compared using a one-way ANOVA, and, as expected from the previous study, the values are not significantly different ($P = 0.35$; Fig. 5*C*). Thus RF size was not altered by partial SC ablation, confirming that afferent/target convergence ratios are conserved at the single SC cell level.

If NMDA receptors are involved in conservation of RF size in the compressed maps, as well as regulating single neuron convergence ratios in normal maps, then NMDA receptor blockade should result in even larger RFs in lesioned animals than in the D-APV group

animals. We found that partial SC ablation in combination with blockade of NMDA receptors (PT/D-APV group) caused a further increase in RF diameter, to nearly twice that measured in normal animals, and on average 19% greater than that seen in the D-APV group (Table 1, Fig. 5, *A* and *B*), consistent with a further increase in convergence of retinal afferents onto single SC neurons. The difference in the distributions of RF size was significantly different from that in both the normal and D-APV groups ($P < 0.001$; Fig. 5*C*). This result supports the hypothesis that NMDA receptors facilitate the ability of the SC cell to regulate the number of afferents it receives from the retina to preserve RF properties.

RF size is related to lesion extent

If APV is blocking the process whereby the SC compensates for the lesion, then larger lesions should produce a greater increase in RF size. The extent of the remaining superficial gray layer of the SC in PT animals ranged from 30 to 70% of normal volume with a mean of $50 \pm 5.9\%$ ($n = 6$). In the PT/D-APV animals, these values ranged from 40 to 90% with a mean of $57 \pm 5.0\%$ ($n = 10$). To determine whether RF size varied linearly with lesion extent, we examined data from PT and PT/D-APV animals with different lesion extents.

As reported in the previous study (Pallas and Finlay 1989), we found that in PT animals without drug treatment, RFs were approximately the same size regardless of lesion extent (Fig. 6; $r^2 = 0.407$). Furthermore, RF sizes in the PT group fell within the variability seen in normal and L-APV-treated animals. However, for PT/D-APV animals, not only are RFs much larger than normal, but the field size is linearly related to lesion extent such that animals with larger lesions (and thus smaller colliculi) have larger single-unit RFs in the SC than animals with smaller lesions (lesion range 10 to 60% SC loss, $r^2 = 0.898$). This observation further supports our hypothesis that NMDA receptor blockade prevents the lesioned SC from compensating for the loss of area and thus prevents the conservation of RF size seen in PT animals.

Specificity of NMDA receptor blockade

In evaluating the role of NMDA receptors in map compression and RF plasticity, a potential concern was that the NMDA receptor blockers delivered by the Elvax might also be blocking other receptors. Thus a series of control experiments using iontophoretic application of glutamate agonists and antagonists was conducted. In vivo iontophoresis of 50 mM D-APV during SC neuron recording in a normal adult hamster blocked much of the response to iontophoretically applied NMDA (Fig. 7, *A* and *D*, Table 2; 74.2% mean reduction in number of NMDA-induced spikes, $n = 3$ units) but had no significant effect on the response to AMPA ($n = 3$ units, $P > 0.4$; Fig. 7, *B* and *D*, Table 2), indicating that the drug was specific for the NMDA receptor even at much higher concentrations than available from the Elvax implants. Application of CNQX reduced the AMPA responses substantially, as expected (AMPA: 77.8% reduction; $n = 3$ units, $P < 0.01$; Fig. 7, *B* and *D*, Table 2), but there was no significant decrement in the NMDA response with CNQX iontophoresis ($P > 0.5$, $n = 3$ units; Fig. 7*A*). The data are summarized in Fig. 7*D* and Table 2.

AMPA receptors underlie most of the glutamate response

If NMDA receptors carry a substantial portion of the glutamate current in the retinocollicular pathway, then NMDA receptor blockade might significantly impair synaptic transmission. To investigate this possibility, iontophoresis of glutamate in the presence of CNQX and/or D-APV was used (Fig. 7, *C* and *D*, Table 2, $n = 3$, the same units were used for each series of drug applications). There was little if any effect of D-APV on the response to glutamate (12.8% mean reduction in response, $P > 0.4$). In contrast, CNQX alone produced a major decrement in the response to glutamate (79.2% reduction, $P < 0.01$), and iontophoresis of both CNQX and D-APV at the concentrations used here nearly eliminated the response to glutamate (96.5% reduction, $P < 0.005$) as expected. These results suggest that NMDA receptors were responsible for only a small proportion of the total glutamate-induced response in these hamster SC neurons as reported by other investigators in rat and guinea pig SC (Binns and Salt 1998a,b; Okada and Miyamoto 1989; Roberts et al. 1991) and in the hippocampus (Clark et al. 1994; Collingridge et al. 1983; Müller et al. 1988).

Effectiveness of D-APV in Elvax implants

To determine the extent to which long-term treatment of the SC with D-APV in Elvax effectively blocked NMDA receptors, we compared the response of SC neurons in normal and D-APV-treated hamsters to iontophoretically applied NMDA ($n = 2$ animals, 3 units per animal; Fig. 8, Table 3). Although the response of SC neurons to glutamate and AMPA was comparable between normal and D-APV-implanted hamsters ($P > 0.4$ for glutamate; $P > 0.7$ for AMPA), the response to NMDA was substantially reduced (although not eliminated) in the D-APV animals compared with normals ($P < 0.03$). It is important to note that these were adult hamsters that were implanted as neonates, that the Elvax had been pushed posteriorly by the growing cortex, and that the Elvax had been removed several hours prior to recording, yet we still observed a substantially reduced NMDA response in the treated tissue. One possible explanation is that there may be APV left behind in the tissue some hours after the implant is removed. Another possibility is that NMDA receptors are downregulated after postnatal treatment with APV. We did not pursue this interesting observation further.

In another adult hamster implanted with D-APV in Elvax from birth, we succeeded in recording immediately after the Elvax was removed (not shown). Comparison with normal animals showed that the responses of SC neurons to iontophoretically applied NMDA were much reduced in this implanted animal also, again suggesting that the implants were effective in blocking much of the NMDA-R current throughout the development of the SC.

Effect of chronic NMDA receptor blockade on visual responsiveness

As noted in the preceding text (Fig. 7, *C* and *D*), NMDA receptors account for only a small component of the total glutamate-evoked response in superficial SC neurons in normal animals. The next series of control experiments tested whether visual responses or the effectiveness of glutamate antagonists in blocking visual responses were altered by the chronic D-APV implantation. These experiments combined visual stimulation in vivo and iontophoretic application of glutamate receptor antagonists (Fig. 9, Table 3; $n = \pm$ units in normal, 3 units in D-APV animals). The Elvax was removed just prior to recording in each case.

Strong visual responses could be recorded from SC neurons in adult hamsters that had Elvax with D-APV implanted since postnatal day (P) 1 (Fig. 9, *A*, *C*, and *E*). There was no significant difference in visual responsiveness in units from normal animals compared with units from chronic APV-treated animals ($P > 0.7$; Fig. 9*F*). In both normal and chronic D-APV group animals, application of CNQX profoundly and reversibly reduced the visual responses (normal: $P < 0.005$; D-APV: $P < 0.02$; Fig. 9, *B* and *F*). The mean extent of the reduction was more severe in the D-APV group, although not significantly so ($P > 0.06$). This is expected because their chronic APV treatment has eliminated much of the NMDA receptor-mediated response that would remain in the absence of AMPA receptor function. Iontophoretically applied APV reduced the responses to visual stimulation somewhat at this high concentration of iontophoretically applied D-APV (50 mM), but the reduction was significantly different from the response to visual stimulation alone only for the normal group (normal: $P < 0.04$; D-APV: $P > 0.2$; Fig. 9, *D* and *F*). The effect was similar between groups ($P > 0.5$; Fig. 9, *D* and *F*), suggesting that AMPA receptor function is not altered by the chronic NMDA receptor blockade.

To more carefully assess the effect of NMDA receptor blockade immediately after implantation, we examined the effect of acute implantation of D-APV in Elvax in three units. A visual response comparable to that in normal animals ($P > 0.4$) was still present 2 days after implantation of D-APV in Elvax in adult hamsters, followed by removal of the implant just prior to recording (Fig. 10, *A* and *B*). This response was not significantly affected by iontophoresis of additional D-APV ($P > 0.3$; Fig. 10*B*).

We were able to examine the effectiveness of visual stimulation in three early postnatal animals between ages P15 and P21 (eye opening is during the 3rd postnatal week). As others have reported, we found that visual responses at this age were weak and difficult to elicit. In one hamster pup implanted with D-APV in Elvax at birth and recorded at P19, 2 days after eye opening, visual stimulation produced a vigorous response (Fig. 10*C*) that was not significantly reduced by iontophoretic application of additional D-APV ($P > 0.09$; Fig. 10, *D* and *E*). This was also true at P15 ($n = 1$; Fig. 10*E*). Thus D-APV treatment leaves effective transmission of visual information intact in young animals just after eye opening when the map refinement process is likely to be in full swing. It is important to note that the dose of the iontophoretically applied D-APV in Fig. 10*D* is much higher than the dose supplied in the Elvax, and thus the visual response is likely to be even more robust in the Elvax-implanted animals.

DISCUSSION

The experiments presented here addressed the role of NMDA receptors in the normal development of the mammalian retinocollicular system and their role in a form of developmental plasticity called map compression. We found, using physiological methods, that the cues that normally guide the initial formation of a normal as well as a compressed retinotopic map are not disrupted by NMDA receptor blockade; NMDA receptors are required for the normal developmental processes of map refinement and the control of afferent/target convergence at both the single neuron and the population level; NMDA receptors are necessary for the conservation of RF size observed in single cells within

compressed maps of PT animals (Pallas and Finlay 1989); and the effect varies linearly with lesion size. Thus formation and compression of the retinocollicular map as a whole are mediated by an NMDA receptor-independent process, whereas an NMDA receptor-dependent process controls the construction of RFs in individual SC neurons. However, retinotopic map formation and receptive field construction must interact in a seamless fashion, even when they are placed in conflict by afferent/target mismatches in the map compression paradigm. It appears that NMDA receptors play a role in coordination of these two processes not only during normal development, but also in compensation for perinatal brain damage.

Methodological considerations

We demonstrated that the 10 mM D-APV/Elvax implants were effective in blocking the NMDA current in both adult and early postnatal animals. Further, doses of D-APV larger than those used in the implants were specific for the NMDA receptor and did not affect the AMPA response. This is consistent with other reports that D-APV is a highly selective antagonist of the NMDA receptor and that at the 10 mM doses used in these experiments, blockade of non-NMDA glutamate receptors is quite unlikely (Artola and Singer 1987; Bear et al. 1992; Huettner and Bean 1988; Mayer 1994; Miller et al. 1989; Ramoa et al. 1990; Woodruff et al. 1987).

NMDA receptors play a specific role in developmental plasticity

NMDA receptors are thought to perform an essential role during normal development in sensory systems. Examples of normal developmental processes that can be prevented with NMDA receptor blockade include topographic refinement of the retinotectal map in frogs (Cline and Constantine-Paton 1989; Scherer and Udin 1989), fish (Schmidt 1990), and rats (Simon et al. 1992) and the alignment of the visual and auditory maps in the optic tectum of ferrets (Schnupp et al. 1995).

Several previous studies have suggested that NMDA receptor-dependent LTP (see Malinow 1994 for review) underlies synaptic plasticity during development as well as in adult learning. In “three-eyed” frogs grafted with an ectopic eye during development, the segregation of synaptic terminals from each eye is NMDA receptor dependent (Cline and Constantine-Paton 1989; Cline et al. 1987). In kittens, the ocular dominance shift that occurs when one eye is covered during development can be prevented by NMDA receptor blockade (Bear 1997; Kleinschmidt et al. 1987). In the somatosensory cortex of the rat, although the “barrels” representing each whisker can form under activity blockade, NMDA receptor antagonists prevent the change in the cortical map caused by removing a whisker row (Schlaggar et al. 1993).

Despite these many examples, a persistent problem in demonstrating that NMDA receptors play a specific role in mammalian developmental plasticity has been that in most paradigms studied to date, blocking NMDA receptors severely compromises or even prevents synaptic transmission (Kasamatsu et al. 1998; Miller et al. 1989; Schlaggar et al. 1993). If NMDA receptor blockade blocks normal activity in addition to blocking the plasticity, one cannot argue that the receptor is playing a specific role because blockade of activity in general also

prevents most forms of plasticity (Cline 1998; Constantine-Paton and Cline 1998; Fox and Daw 1993). In some novel experiments using anti-sense oligonucleotides (Roberts et al. 1998) or low doses of MK-801 (Daw et al. 1999), partial loss of NMDA receptor function prevented ocular dominance plasticity without completely blocking synaptic transmission, providing data supportive of a specific role for NMDA receptors. In some cases (e.g., Simon et al. 1992), this issue has not been addressed and would be of great interest.

In this study, we demonstrated that NMDA receptors are not necessary for normal visual responses in hamster SC. We demonstrated that acute or chronic blockade of NMDA receptors left the synaptic responses elicited by visual or pharmacological stimulation intact. Also as was shown in other rodents (Hestrin 1992; Okada 1993), the NMDA receptor carries only a small proportion of the total glutamate-induced current in hamster SC. Thus the map compression paradigm in hamster SC allows us to draw conclusions regarding a specific role for NMDA receptors in developmental plasticity.

NMDA receptor blockade has no effect on map topography

We found that retinocollicular map topography was unaffected by NMDA receptor blockade, in either normal or compressed maps, with no reversals or losses of visual field representation within the map. Given the results of Simon and colleagues demonstrating substantial mistargeting of rat retinal axons after long-term NMDA receptor blockade (Simon and O'Leary 1992; Simon et al. 1992), this result was surprising. The anatomical data of Simon and colleagues predicted more disorder in the map than we saw with our physiological techniques. It is possible that our technique could not reveal such map errors, that they would have been corrected prior to adulthood, or that the mistargeted projections were suppressed physiologically. This issue might be resolved with a more finely detailed mapping experiment.

We also found that NMDA receptor-based activity was not necessary to compress the retinotopic map in SC. A previous study in fish using TTX to block activity suggested that compression and expansion of retinotectal maps is activity independent in regenerating retinotectal projections (Meyer and Wolcott 1987) (note the use of TTX may be problematic because it may promote axonal sprouting). Whether map compression requires or drives a redistribution of the molecular markers responsible for establishing the initial map, or whether they are ignored, is an important question that we are currently addressing.

Our results emphasize the importance of cooperation between activity-dependent and -independent developmental processes in neural patterning and circuit formation. Recent work on developing visual cortex has suggested that ocular dominance columns can form in the absence of the eyes (Crowley and Katz 1999, 2000), but maintenance (Chapman 2000) as well as plasticity of the columns during a critical period (Issa et al. 1999) depends on neuronal activity. A similar scenario is evident in the formation of orientation-specific circuitry in visual cortex (Chapman et al. 1999; Crair et al. 1998; Ruthazer and Stryker 1996). It is increasingly becoming apparent that there is a substantial degree of early bias in intrinsic patterning during brain development, during which extrinsic influences are either not present or are ignored. This stage is followed by a later stage during which lack of normal input leads to adjustment of the circuits (plasticity) in a way that optimizes

functional capacity according to what type of anomalous input is available. This dual cooperative process ensures development within normal parameters while retaining the flexibility to adapt to changing, unexpected conditions.

NMDA receptor blockade increases RF sizes of SC neurons in normal and compressed maps

The RF size of visual neurons in the brain is in large part dependent on the number of retinal inputs they receive and the location of the inputs relative to the visual field representation (Chung and Ferster 1998; Ferster 1994; Hubel and Wiesel 1962). A mapping process that worked by a simple competition for target space would result in RF sizes of individual target cells being determined by the number of afferents available to each target cell and the number of axons they extended regardless of the size of the target. In this case, map compression would result in increased RF sizes and decreased acuity. Developmental processes do not initially generate input and target neurons in matching quantities (Finlay and Pallas 1989 for review) and often more than one afferent population must map onto the same target (e.g., Schnupp et al. 1995), increasing the difficulty of the matching process. Previous results demonstrated that the retinocollicular system can effectively compensate for a wide range of afferent/target mismatches, maintaining receptive field properties and thus proper visual function (Pallas and Finlay 1989). The results presented here show that this is likely to occur through the special properties of the NMDA receptor.

Simon and colleagues noted on the basis of anatomical studies that NMDA receptor blockade produced not only mistargeting of retino-SC axons, but increased axonal side-branching (Simon et al. 1992). Increased branching might be expected to result in larger and more irregular receptive fields in SC neurons. Our physiological data suggest that this is the case. The RF borders of units from the D-APV and PT/D-APV groups were more irregular and diffuse than in the normal, L-APV, or PT groups (cf. Fig. 3), suggesting that they receive input from widely scattered ganglion cells. Furthermore NMDA receptor blockade resulted in significantly larger RFs in these units, especially within compressed maps. These observations provide physiological support for the idea that NMDA receptors regulate convergence of visual input onto single target cells, matching afferent and target populations even under conditions of increased afferent availability. We also found that the effect was related to lesion size. This was predicted from our previous anatomical results in PT animals, showing that the number of retinal ganglion cells innervating a unit of SC is constant for losses of up to approximately 70% of the SC (Pallas and Finlay 1991), a compensatory process that occurs via regulation of retinal axon arbor morphology (Xiong et al. 1994).

Previous results led us to propose that NMDA receptors act as coincidence detectors with a threshold, preventing excess available retinal inputs from stabilizing synaptic contacts unless the level of coincidence in their activity, and thus their relative position in the retina, falls within a certain spatiotemporal window (Finlay and Pallas 1989; Pallas and Finlay 1989). The data from the present study support this interpretation. Recent studies in tadpoles (Zhang et al. 1998) have directly supported the existence of a narrow time window within which cooperating inputs must be co-active to stabilize connections on a common target

neuron and exclude competing inputs. NMDA receptor blockade in the continually shifting retinotectal map in frogs results in progressively more mistargeted retinal axons in tectum (see Cline 1991 for review) as would be expected with the time window hypothesis.

Our current working model is as follows (Fig. 11): Early in development, retinal axons project widely, such that each SC neuron receives input from many retinal ganglion cells (Fig. 11A, *left*). Normally the retinal axon arbors are pruned through NMDA receptor-directed map refinement, such that each SC neuron receives convergent input from very few retinal neurons (Fig. 11A, *right*). In compressed maps, the ability of NMDA receptors to detect not only *whether* competing inputs exhibit correlated activity but *how* correlated they are in an absolute sense, maintains the single-unit retinocollicular convergence ratio through both reductions in the extent of retinal axon arbors and increased divergence of retinal axons to alternate targets (Pallas and Finlay 1991; Xiong et al. 1994) (Fig. 11B). If NMDA receptors are blocked during this process, not only is the normal map refinement process blocked (Fig. 11A, *bottom*), but so is the compensation process that operates in compressed maps (Fig. 11B, *bottom*), leading to further lesion size-dependent increases in single unit RF size (*far right*, 75% lesion).

We suggest that our data show an increased convergence of retinal afferents onto single SC neurons resulting from NMDA receptor blockade. Our results strongly suggest that NMDA receptors provide the means for the retinocollicular system to compensate for mismatches in afferent/target ratios by controlling convergence at the single cell level and thus play an essential role in the construction and conservation of RF properties that occurs in normal and compressed maps.

Relevance to other studies

The strongest evidence for a specific coincidence-detection role for NMDA receptors in visual system plasticity has been obtained from the frog retinotectal system (Cline and Constantine-Paton 1989; Cline et al. 1987; Scherer and Udin 1989; Udin and Grant 1999; Udin et al. 1992). Our results show that the mammalian homologue of the optic tectum, the superior colliculus, also provides a good model system for studies of developmental plasticity in the visual system. Our demonstration that blockade of NMDA receptors preserves visual responses in SC suggests that this may also be true in other mammalian visual nuclei, such as LGN, which has also been the subject of much research on the role of NMDA receptors in visual system development and plasticity (Cramer and Sur 1996; Hahm et al. 1991, 1999).

NMDA receptors are undoubtedly not the only means of coincidence detection that neurons have at their disposal. There are forms of LTP known to be NMDA receptor-independent (e.g., Nicoll and Malenka 1995; Teyler et al. 1995), and there is evidence in the ocular dominance plasticity paradigm for an incomplete dependence of *in vivo* plasticity on common forms of LTP (Gordon et al. 1996; Hensch et al. 1998) and on Hebbian learning (Crair et al. 1998; Ruthazer et al. 1999). Other forms of LTP also employ calcium-triggering of signal transduction mechanisms; thus there are multiple entry points into this signal transduction pathway, activation of NMDA receptors being only one. It will be necessary to

identify these other pathways to acquire a more complete picture of the mechanisms underlying developmental plasticity.

Implications for development and evolution of the visual system

The ability of the visual system to compensate for severe damage is remarkable. In this and our previous work in hamster SC, we demonstrated that removal of more than 50% of the SC did not adversely affect retinocollicular topography or SC neuron receptive field properties. The present results showing that NMDA receptors mediate this compensation process may provide insight into strategies for clinical intervention after perinatal brain damage. Furthermore they provide additional evidence of mechanistic commonalities between developmental plasticity and adult learning (Constantine-Paton and Cline 1998).

Developmental mechanisms have often been co-opted for evolutionary changes in brain structure and function (Goodman and Coughlin 2000; Pallas 2001a,b). The ability of the brain to match its components and preserve function even in cases where the components are mismatched in number could provide an important substrate for brain evolution. Slight alterations in NMDA channel kinetics or the integrative properties of neurons could translate into alterations in afferent/target convergence ratios. This could provide the means to emphasize or de-emphasize certain portions of the visual field. In fact, there is a general phylogenetic trend among vertebrates for increased emphasis of central visual field, and a comparative study could provide insight into the underlying mechanism.

In conclusion, our results strongly suggest that NMDA receptors provide the means for the retinocollicular system to compensate for mismatches in afferent/target ratios by controlling convergence at the single-cell level and that they play an essential role in the construction and conservation of RF properties under conditions of normal development and perinatal brain damage. However, they are not necessary for wholesale formation of topographic maps, even if the target area is substantially altered in size. Our results support the idea that NMDA receptors in concert with the integrative properties of the target neurons provide a means whereby the absolute number and relative RF location of competing inputs can be regulated to preserve perceptual acuity. This is particularly important in topographically mapped systems but is likely to be of general importance throughout the nervous system.

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FIG. 1.

D-APV release from Elvax as calculated from scintillation counts. The Elvax slices were preincubated in PBS for 48 h before implantation to avoid exposing the superior colliculus (SC) to the initial burst of release (○). In = cases, the phosphate-buffered solution (PBS) was changed, and the Elvax was incubated an additional 48 h (●). These = slices were not implanted, but they revealed that the early release immediately after implantation is much reduced by preincubation. At least 3 mo after implantation and just prior to physiological recordings, Elvax pieces were removed for measurement of the release rate (▼). Release was near 0 by 12 mo postimplantation.

FIG. 2.

Regularity of map topography. The progression of nasotemporal receptive-field locations with rostrocaudal SC recording site location is plotted for representative animals in each relevant group. The plots show that there is a regular progression of visual field location with SC recording site location. The numbers at the *top*, as indicated in the legend, mark the caudal limit of the SC in each animal. The proportional size of the remaining SC in the lesioned animals is also presented in the legend. Note that the animals with larger lesions (5 and 6) have more compressed maps, irrespective of treatment with *N*-methyl-D-aspartate (NMDA) receptor antagonists. r, rostral; c, caudal; N, nasal; T, temporal.

FIG. 3.

Receptive field plotting methods. *A*: hand plots were translated from a translucent hemisphere into 2 dimensions via polar coordinates. The optic disk is in the center of the plot. Shown are representative fields (RFs) plotted from 1 animal within each group. Only nasocentral units were used in the quantitative analysis. Note the variation in RF size with eccentricity and with experimental treatment. (N, nasal; T, temporal; U, upper; L, lower visual field.) *B*: method of computer-aided receptive field plotting. One example is shown for 3 experimental groups. A small spot of light was swept vertically and bidirectionally

across the computer screen. Naso-temporal RF diameters were used for all quantitative analyses.

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FIG. 4.

Responsiveness to visual stimulation from single units in each experimental group. The graph shows responsiveness, in number of action potentials per stimulus, of units whose responses were digitized from each group of animals (computer-plotted units). Each point shows the response of 1 unit to 1 computerized stimulus presentation. Points aligned vertically are from the same animal. Statistical comparison of the range and distribution demonstrated that the experimental treatments had no significant effect on excitability levels.

FIG. 5.

Comparison of RF diameters. *A*: cumulative percentage distribution of RF diameters for all single units in each group of animals. Each point represents the percent of neurons with RF diameters of that size and smaller. Note the overlap in RF size distribution in the normal, PT, and L-APV groups, and the absence of overlap between those 3 groups and the D-APV and PT/D-APV groups. In particular, it can be seen that the PT/D-APV group has a number of neurons with very large RFs. *B*: histogram of means and SEs for each group of animals. Data were compared with *n* as the number of animals rather than the number of neurons. *, significance as compared with the normal group. The mean RF size of the D-APV group was larger than that of the normal, PT, or L-APV group, suggestive of blockade of map refinement. The RFs of the PT/D-APV group were in turn significantly larger than those of the D-APV group, suggesting that blockade of NMDA-R prevents not only map refinement

but also compensation for the increased afferent/target ratios. *C*: raw data showing the RF diameters for the entire data set. Note that the drug treatment shifts RF size in the entire population.

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FIG. 6.

Effect of lesion size on RF size. The residual volume of the SC is presented in a declining direction on the *x* axis so that data from normal, full-sized colliculi are plotted on the *left* with open symbols, and larger lesions (smaller SC) are plotted with closed symbols on the *right*. The mean RF size of each unlesioned animal is plotted at 100% SC volume, and the mean of the means (shown by $\rightarrow$) is the origin of the 2 regression lines. The increase in RF size seen in PT/D-APV animals was linearly related to lesion size ($r = 0.898$, $n = 10$), with a greater increase in single-unit RF size resulting from larger lesions. In PT animals, RF size was unrelated to lesion size ($r^2 = 0.407$, $n = 6$), suggesting compensation for the afferent/target mismatch.

FIG. 7.

Specificity of glutamate receptor blockers. *A–C*: drugs were iontophoresed from multi-barrel pipettes while recording single-unit activity through a 3 M NaCl-filled barrel. Each record represents the response of 1 representative unit to 1 trial. Duration of drug application is indicated by the horizontal bar under the *x* axis. Bin size is 2 s. *A*: D-APV (center) blocked the response to NMDA (*left*). Application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) produced an intermediate effect (*right*) probably due to the dependence of the NMDA channel on the AMPA current. *B*: CNQX (*right*) but not D-APV (*middle*) blocked the

response to AMPA (*left*). *C*: CNQX and APV together (*right*) blocked the response to glutamate (*left*). CNQX (*2nd from right*) produced a much larger decrement in the glutamate response than APV alone (*2nd from left*), indicating that most of the glutamate current is carried by the AMPA channel. *D*: graph showing the responses of all cells tested with the agonists glutamate, AMPA, and NMDA. *, significance in comparison to each agonist. Combined APV and CNQX blockade was tested only with the glutamate trials. Note that APV has little effect on the response to glutamate compared with the effect of CNQX but does have a major effect on the response to applied NMDA.

FIG. 8. Effectiveness of long-term D-APV treatment for NMDA-R blockade. Drugs were iontophoresed from multi-barrel pipettes while recording single-unit activity through a 3 M NaCl-filled barrel. Each record shows the response of one representative unit to 1 trial. Bin size is 2 s. *A*: response of 1 SC neuron from a normal adult to glutamate, AMPA, and NMDA. *B*: treatment with D-APV in Elvax from postnatal day 1 to adulthood caused a decrement in the response to NMDA compared with normal, but the glutamate and AMPA responses are similar, indicative of an effective block of the NMDA receptor with the chronic D-APV implants. *C*: summary of the responses of all units tested in this control experiment. Note that the NMDA (*) but not the AMPA or glutamate response is significantly curtailed in the APV-implanted animals. (*, comparison between normal and chronic D-APV response to NMDA.)

FIG. 9.

Effect of NMDA and AMPA receptor blockade on visual responsiveness. Drugs were iontophoresed from multi-barrel pipettes while recording single unit activity through a 3 M NaCl-filled barrel. During the recording, three consecutive visual stimuli were presented. Bin size is 0.2 s. *A–E*: time series recordings from a chronic D-APV implanted adult hamster. Each record shows the response of 1 representative unit to 1 trial. Long-term D-APV treatment did not prevent a strong response to visual stimulation (*A*, *C*, and *E*). The visual response was affected severely by application of CNQX (*B*) but only partially by application of additional D-APV (at a far higher dose than that supplied by the implant; *D*). *F*: summary of data obtained from adults treated with long-term APV implants. The data from *A*, *C*, and *E* are combined (visual stimulation). The magnitude of the visual response in the D-APV-implanted animals is comparable to normal (see Fig. 4), but is reduced with

application of additional APV in both normal and implanted animals. Applied CNQX nearly eliminates the response (*), reflecting the greater contribution of AMPA receptors to the visual response. See text for additional statistical comparisons.

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Acute APV, Adult **Neonatal APV** **E Summary** **FIG. 10.**

A: there was also a strong visual response from SC neurons after acute (2 day) treatment with D-APV. *B*: application of additional D-APV reduced the visual response somewhat. *C* and *D*: animals with D-APV applied from birth until $\pm$ days after eye opening (P19) also respond to visual stimulation, but iontophoretic application of additional D-APV decreases the response more than in the adults (*D*). *E*: summary of data from acutely treated adults and implanted P15 and P19 animals. Note that the level of response to visual stimuli is similar to that seen in normal animals (Fig. 5). See text for statistical comparisons.

FIG. 11.

Proposed mechanism for changes in connectivity patterns resulting from early PT and/or D-APV treatment. The model depicts a representative patch of SC. To eliminate edge effects, attention should be directed to the SC cells in the center of each patch. *A*: in normal animals, some collaterals of the neonatal retinocollicular projection are normally eliminated prior to adulthood via the map refinement process. In the model, this results in each SC neuron receiving input from 2 retinal ganglion cells and in each ganglion cell innervating 2 SC cells. *B*: as has been shown (Pallas and Finlay 1989), the RF size of single SC neurons is unaffected by PT lesions, because convergence ratios are maintained through decreased retinal axon arbor extent and selective divergence of retinal axons to other targets. For the D-APV and PT/D-APV groups, we suggest that some exuberant collaterals would be maintained due to inactivation of the coincidence detection mechanism supplied by the NMDA-R. This would cause an increased convergence of ganglion cells onto SC cells, and an enlargement of RF size in the D-APV group due to prevention of map refinement and control of single cell convergence, and a further enlargement in PT/D-APV animals due to the combined effects of blocking map refinement and the mechanism that compensates for mismatches in afferent and target populations. With larger lesions (e.g., a 1-celled SC in the model, representing a 75% loss of SC), convergence, and thus RF size, would be greater.

TABLE 1

Measurements of mean receptive field size of superior colliculus (SC) cells within each group of animals

Group	Animal Data	Single-Unit Data
Normal	9.2 ± 0.40 (8)	9.4 ± 0.29 (40)
PT	10.1 ± 0.17 (6)	10.1 ± 0.28 (35)
L-APV	10.4 ± 0.18 (5)	10.5 ± 0.18 (35)
D-APV	15.1 ± 0.22 (16)	14.9 ± 0.15 (59)
PT/D-APV	18.0 ± 0.59 (10)	18.0 ± 0.31 (58)
Totals	45	227

The data are presented using both individual animals and single neuronal units as observations. Values are means ± SE in degrees. *n* values are in parentheses. PT, partial tectum; L- and D-APV, inactive and active form of amino-5-phosphonovalerate.

TABLE 2

Tests for specificity of glutamate antagonists (see Fig. 7)

Agonist	+APV	+CNQX	+APV/CNQX
Glu: 67 ± 8.7	58 ± 6.6 (12.8)	15 ± 4.4 (79.2)	3 ± 1.5 (96.5)
AMPA: 55 ± 7.9	46 ± 8.4 (17.9)	12 ± 2.7 (78.5)	NA
NMDA: 37 ± 9.0	10 ± 3.1 (74.2)	29 ± 7.9 (20.9)	NA

The response to each agonist (column 1) and to each combination of agonist and antagonist (rows) is presented as the mean $\pm$ SE number of action potentials, with the corresponding reduction of each agonist-induced response indicated in parentheses as a percent block by the antagonist. All data presented in this table were collected from the same group of normal SC cells. NA, not applicable; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; NMDA, *N*-methyl-D-aspartate.

TABLE 3

Effect of D-APV implantation on responses of SC cells to iontophoretically applied glutamate agonists and antagonists and on visual responsiveness (see Figs. 8-10)

Group	<i>n</i>	Total Response	AMPA Response	NMDA Response
Normal	3	Glu: 48 ± 11.1	AMPA: 33 ± 7.7 (70.2)	NMDA: 20 ± 5.4 (42.6)
	6	Vis: 40 ± 6.3	Vis + APV: 22 ± 7.2 (57.2)	Vis + CNQX: 11 ± 1.6 (27.8)
Chronic D-APV	3	Glu: 38 ± 5.8	AMPA: 34 ± 6.6 (88.4)	NMDA: 6 ± 1.9 (16.3)
	3	Vis: 43 ± 8.9	Vis + APV: 27 ± 3.6 (60.4)	Vis + CNQX: 5 ± 1.7 (10.8)
Acute D-APV	3	Vis: 48 ± 8.1	Vis + APV: 38 ± 5.2 (81.2)	NA
Neonatal D-APV	3	Vis: 35 ± 3.8	Vis + APV: 23 ± 3.8 (66.2)	NA

The response to each agonist (Glu, AMPA, NMDA) or to visual stimulation (Vis) and to each combination of visual stimulation and antagonist (Vis + APV or Vis + CNQX) is presented as the mean number of action potentials ($\pm$ SE) and in parentheses as a percent of the glutamate (Glu)-evoked or visual stimulation (Vis)-evoked response. All data presented in each row were collected from the same group of normal SC cells (sample size as indicated).