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NMDA Receptor Blockade in the Superior Colliculus Increases Receptive Field Size Without Altering Velocity and Size Tuning

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Razak, Khaleel A., Lihua Huang, and Sarah L. Pallas. NMDA receptor blockade in the superior colliculus increases receptive field size without altering velocity and size tuning. *J Neurophysiol* 90: 110–119, 2003. First published January 15, 2003; 10.1152/jn.01029.2002. Neonatal brain injury triggers compensatory processes that can be adaptive or detrimental, but little is known about the mechanisms of compensation or how they might affect the response properties of neurons within the injured region. We have studied this issue in a rodent model. Partial ablation of the hamster superior colliculus (SC) at birth results in a compressed but complete visual field map in the remaining SC and a compensatory conservation of receptive field (RF) size and stimulus velocity and size tuning. The circuit underlying stimulus tuning in this system or its preservation after brain lesions is not known. Our previous work has shown that *N*-methyl-D-aspartate (NMDA) receptors are necessary for the development and conservation of RF size after partial SC ablation. In this study, we examined whether NMDA receptor function is also necessary for the development and conservation of stimulus velocity and size tuning. We found that velocity and size tuning were unaffected by chronic postnatal blockade of NMDA receptors and the resulting increases in RF size. Thus NMDA receptors in the SC are not necessary for the development of stimulus velocity and size tuning or in the compensatory maintenance of these properties following brain damage. These results suggest that stimulus velocity and size tuning may arise in the retina or from NMDA receptor-independent circuitry intrinsic to SC. The lack of conflict between NMDA receptor activity-dependent and -independent processes may allow conservation of some RF properties while others change during injury-induced or evolutionary changes in afferent/target convergence.

INTRODUCTION

The anatomy and physiology of visual pathways have been well studied, but surprisingly little is known about how the circuitry for complex receptive field properties is constructed during normal development. Hubel and Wiesel (1962, 1965) originally proposed that some response properties in visual cortex, such as receptive field (RF) size, stimulus orientation tuning, and the simple or complex categories of receptive field substructure, depend critically on summation of the proper number, source, and type of afferent inputs to each target cell. This view has been supported by numerous subsequent studies (Alonso et al. 2001; Chapman et al. 1991; Ferster and Miller 2000; Martinez and Alonso 2001; Roerig and Chen 2002; Usrey et al. 2000). Others have argued, however, that stimulus tuning is shaped substantially by lateral inhibition within cortex (Allison et al. 1996; Crook et al. 1998; Eysel et al. 1998; Shevelev et al. 1998; Sillito 1975). That there is still substantial disagreement about the relative roles of excitatory convergence and lateral inhibition in sculpting visual response properties suggests that insight may be gained by examination of a different model system. In this study, we have employed an experimental procedure in hamsters that increases the degree of convergence to examine whether RF properties in the superior colliculus (SC) depend on the convergence of afferents from retinal ganglion cells.

Another question that can be addressed by our model system is how construction and maintenance of RF properties might be affected by brain damage. The extent to which plasticity after perinatal brain damage is behaviorally adaptive depends on how functional properties of the altered brain region are affected, either by the damage itself or by the compensation process underlying the plasticity (Payne and Lomber 2001). Yet there have been few studies addressing this issue. Because our experimentally induced increase in retinocollicular convergence involves neonatal brain lesions, we can study the effect of increased afferent convergence on construction of RF properties after brain damage.

One major advantage of using hamsters as a model is that the retinocollicular projections form largely after birth, and thus the convergence and map refinement processes can be challenged prior to retinal activation of the SC by manipulations done on the day of birth. Responses to visual stimulation are not present in SC until approximately postnatal day 12, 1 day before natural eye opening (Binns and Salt 1997; Clancy et al. 2001; Huang and Pallas 2001). Experimental manipulation of the degree of convergence in our model system is made possible by an interesting form of plasticity exhibited by the hamster retinocollicular pathway. Partial ablation of the SC at birth results in a compressed yet complete map of visual space within the remaining SC (Finlay et al. 1979). Despite reorganization of retinocollicular connections after the lesion, afferent/target convergence ratios are conserved at the single-cell level. The compensatory nature of the reorganization is reflected in the conservation of normal response properties including RF size, stimulus velocity tuning, and stimulus size tuning (Pallas and Finlay 1989). This remarkable conservation of visual response properties was proposed to result from activity-dependent mechanisms that preserve single neuron convergence ratios (Pallas and Finlay 1991; Xiong et al. 1994).

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N-Methyl-D-aspartate (NMDA) receptors have been proposed to play a role in maintaining afferent/target convergence ratios during normal development in the cerebellum (e.g., Rabacchi et al. 1992) and the thalamocortical pathway (Fox et al. 1996). It has been demonstrated that NMDA receptors are necessary not only for normal map refinement (Huang and Pallas 2001; Simon et al. 1992) but also for the plasticity that conserves RF size in SC neurons within compressed retinocollicular maps (Huang and Pallas 2001). Chronic blockade of NMDA receptors in both intact and partially ablated SC resulted in significantly larger RF sizes compared with normal SC without affecting visual responsiveness. This effect appears to occur through a combined blockade of the normal map refinement and through preventing the compensation for map compression in the partially lesioned SC. These observations suggested that NMDA receptors play a key role in maintaining afferent/target convergence ratios in the SC during normal development and map compression, likely via spike-timing dependent plasticity (Zhang et al. 1998) and competition between afferents for target space (Cline 1998; Debski and Cline 2002). Furthermore, they suggested the possibility that afferent/target convergence ratios could be experimentally manipulated via pharmacological blockade of NMDA receptors, allowing an examination of the extent to which construction of response properties depends on summation of afferent RF properties.

Neurons in the SC are selective for stimulus velocity and size (Chalupa and Rhoades 1977; Finlay and Pallas 1989; Huerta and Harting 1984; Stein and Dixon 1979; Tiao and Blakemore 1976). These properties are thought to arise within the SC and are important for SC mediated behaviors such as visuomotor integration and orientation (Chalupa and Rhoades 1977; Lomber et al. 2001; Sparks et al. 2001; Tiao and Blakemore 1976; Wallace et al. 1998). If spatiotemporal interactions between converging inhibitory and excitatory retinal inputs are responsible for sculpting neural selectivity for stimulus velocity and size in SC as has been proposed (Chalupa and Rhoades 1977; Goodwin and Henry 1978; Stein and Dixon 1979; Tiao and Blakemore 1976; Waleszczyk et al. 1999), then these response properties are likely to be affected by natural or experimentally induced alterations in afferent/target convergence ratios. The present study was initiated to study the dependence of complex RF properties on afferent/target convergence and to determine the role of NMDA receptors in the construction of stimulus velocity and size tuning circuits in the SC of the hamster during both normal development and reorganization after early injury. To test the hypothesis that NMDA receptor-controlled afferent convergence plays a role in creating these response properties in intact and partially lesioned SC, NMDA receptors were pharmacologically blocked from the day of birth, and at adulthood, stimulus velocity and stimulus size tuning of single SC neurons in these experimental groups were compared with those in normal SC. If the construction of complex response properties is dependent on convergence of afferent input and if the convergence ratio depends on NMDA receptor function, then it can be predicted that NMDA receptor blockade would alter those response properties. On the other hand, if response properties do not result from summation of afferent input, increasing the afferent/target convergence by NMDA receptor blockade should not affect stimulus tuning. Moreover, if NMDA receptors are required for the compensatory plasticity that conserves velocity and size tuning in partially lesioned SC, their chronic blockade should prevent not only the normal development of response properties but should also prevent their conservation in compressed maps.

We found that despite significant increases in RF sizes, NMDA receptor blockade in either intact or partially lesioned SC did not alter stimulus velocity or size tuning of neurons. These results suggest that velocity and size tuning of SC neurons are not dependent on convergence of retinal inputs onto single SC neurons. Although NMDA receptors play a role in refinement of RF size in the intact SC and in refinement of RF size and compensation for mismatched afferent/target ratios in the partially lesioned SC, these data argue that they are not involved in the construction or conservation of velocity and size tuning circuitry in either developmental scenario. This independence from NMDA receptor activity may facilitate preservation of function after SC damage, while allowing topographic maps to conform to changing convergence ratios during either development or evolution.

METHODS

Animals

Forty-two 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 Laboratories (Wilmington, MA). Normal animals were either purchased as adults or bred in the colony. All animal procedures were approved by the Institutional Animal Care and Use Committee, and met standards of accepted care developed by the National Institutes of Health, the American Physiological Society, and the Society for Neuroscience.

Experimental design

Three groups of animals were used. The normal (N) group ($n = 18$ animals) received neither surgical nor drug treatment. The D-2-amino-5-phosphonovaleric acid (D-APV) group ($n = 14$ animals) had the biologically active form of the NMDA receptor antagonist D-APV (Tocris Neuramin, Langford, UK) in Elvax polymer (DuPont, UK) implanted over the SC at birth and throughout postnatal development. This group was designed to determine if NMDA receptors play a role in sculpting stimulus velocity and stimulus size tuning of SC cells during normal development. In the PT/D-APV group ($n = 10$ animals), D-APV treatment was combined with heat cauterization of the caudal part of SC at birth. This group was included to determine if NMDA receptors play a role in the maintenance of stimulus velocity and stimulus size tuning under increased afferent availability after partial SC ablation (Pallas and Finlay 1989). After rearing the hamsters to adulthood under pharmacological blockade of NMDA receptors, response properties were assessed using in vivo extracellular single-unit recording.

Elvax preparation

The D-APV impregnated Elvax polymer was generously donated by Dr. Adam Smith (University of Oxford, UK) and was prepared according to published methods (Schnupp et al. 1995; Silberstein and Daniel 1982; Smith et al. 1995). The polymer was prepared to contain a final concentration of 10 mM D-APV, and a small amount (1: 100,000) of tritiated APV to provide a measure of drug release rate. The initial procedures prior to implantation and the drug release characteristics of the polymer have been reported previously (Huang

and Pallas 2001). Briefly, 100- to 200-μm-thick Elvax sheets were preincubated for 48 h in phosphate-buffered saline (PBS; pH 7.4; 0.5 ml) to prevent exposing the SC to an initial burst of drug release. The Elvax was then inserted under the skull and over the SC in the experimental animals. On implantation on the surface of the SC, the polymer continues to release the drug at a gradually declining rate for $\leq$12 mo (Huang and Pallas 2001; Smith et al. 1995). We have demonstrated that this Elvax preparation successfully blocks a substantial proportion of the NMDA receptor-dependent glutamate component of retinocollicular transmission, without reducing the AMPA receptor-dependent component (Huang and Pallas 2001). The same Elvax preparation was used in both studies.

Surgical procedures

Neonatal surgery was performed within 12 h of birth. Hamster pups were initially anesthetized with 4% isoflurane in 0.5 l/min oxygen and then maintained in a deep surgical plane of anesthesia with 1–2% isoflurane. For the D-APV group, an incision was made through the skull at the boundary between the SC and the inferior colliculus, and a sheet of Elvax was cut to fit and slipped under the skull and over the right SC. For the PT/D-APV group, the superficial layers of the caudal portion of the right SC were ablated after insertion of the drug-impregnated Elvax. The pups were returned to maternal care after closure of the wound and recovery from anesthesia. All subsequent procedures were done at adulthood ($\geq$3 mo of age).

Adult hamsters were prepared for physiological recordings by anesthetization with urethan (0.7 g/ml ip; 0.03 ml/kg in 3–4 aliquots spaced at 20-min intervals). The pupils were then dilated with a 10% ophthalmic atropine solution. Respiration rates and withdrawal reflexes were monitored to ensure a deep level of anesthesia appropriate for surgery, with supplemental doses of urethan given as needed. After performing a craniotomy over the SC, the sagittal sinus was ligated and cut. The visual cortex was bilaterally aspirated to eliminate influences of corticocollicular projections (Rhoades and Chalupa 1976, 1978) and to facilitate viewing the surface of the SC for electrode placement. For the experimental groups, the position of the Elvax strip was noted prior to removing it for scintillation counting of residual drug content. The brain was kept covered with sterile saline. To stabilize the eye during the recording session, the conjunctiva at the nasal corner of the left eye was anchored by a suture to a stereotaxic frame in which the hamster's head was held. The eye was covered with a fitted plano contact lens for protection during the recording session.

Electrophysiological recording

After removal of the Elvax polymer, recording sessions commenced with the determination of the position of the optic disk with a reversing ophthalmoscope. For extracellular single-unit recording, tungsten microelectrodes (FHC, Bowdoinham, ME) with a tip diameter of 1–2 μm and impedance of 1–3 MΩ were used. Using a penlight as a search stimulus, electrode penetrations were made perpendicular to the surface of the SC to locate visually responsive cells in the retino-recipient superficial gray layer (SGS) (Pallas and Finlay 1989). A rapid multi-unit mapping of the rostrocaudal extent of the SC was performed in both D-APV and PT/D-APV cases to ensure that the entire visual field was represented in the SC. Only those animals with a full representation of the visual field within the right SC (the side of experimental manipulation) were studied further. Once the mapping was complete, electrode penetrations were targeted to isolate single units residing within 100 μm of the SC surface and with RFs centered within 15° of the optic disk. This region of the SC exhibits regular compression of the retinal representation after partial SC ablations (Finlay et al. 1979). Moreover, restricting the recording sites to this region reduced the likelihood that differences in response properties at different retinal eccentricities affected comparisons across the three groups of animals (Fortin et al. 1999; Tiao and Blakemore 1976).

Visual stimulation and response selectivity

The location of the excitatory RF (eRF) of each neuron was first determined using a penlight. A 14-in computer display monitor was then placed 40 cm in front of the hamster's eye such that the neuron's eRF was in the center of the monitor. A Sergeant Pepper graphics board (Number Nine, Cambridge, MA) was used in conjunction with "STIM" software (developed by K. Christian and Rockefeller University) to generate visual stimuli consisting of single, smoothly moving light spots that could be varied in diameter, direction, and velocity. A minimum intertrial interval of 5 s was used to prevent adaptation. Data were acquired by CED 1401 hardware and processed by Spike2 software (Cambridge Electronic Design, Cambridge, UK). The nasotemporal diameter of the eRF was determined by sweeping 1° spots of light from the top to the bottom of the monitor screen at different nasotemporal locations with an interstimulus distance of 2° of visual field. The choice of stimulus velocities and sizes used in this study was guided by previous results showing that the majority of hamster SC neurons are selective for small ($<$7.5°) slowly moving ($<$10°/s) stimuli (Stein and Dixon 1979; Tiao and Blakemore 1976). Neural selectivity for stimulus velocity was determined by sweeping a 2.5° diam spot of light in a temporal to nasal direction through the center of the eRF, using velocities from 5 to 45°/s increasing at 5°/s intervals. Selectivity for stimulus size was determined by sweeping a spot of light through the center of the eRF with a velocity of 5°/s and ranging in size from 2.5 to 15° in diameter at 2.5° size intervals. Each stimulus set was repeated at least five times, and the responses were expressed as the mean ($\pm$SE) number of spikes for each stimulus velocity or size. The location of the eRF was re-plotted after the determination of size and velocity tuning to ensure that the entire stimulus set remained centered on the eRF. The response selectivity curve for each neuron was normalized to the response elicited by the best stimulus to facilitate comparison across the three groups of animals, independent of variations in response magnitude between neurons. The best stimulus velocity or size was defined as one that not only elicited the maximum response from the neuron, but also elicited a response that was at least two times the response to the least preferred stimulus. If a continuous range of stimulus velocities or sizes satisfied these criteria, the best stimulus was noted as the middle value of the range. Neurons were also classified according to the profile of velocity or size selectivity curves as low-pass, band-pass, high-pass, and nonselective. A neuron was categorized as low-pass in its velocity tuning if its response to a stimulus moving $<$15 °/s was at least twice that of the least preferred stimulus. High-pass neurons were those that responded strongest to stimuli moving $>$25 °/s, and had $>$50% of their maximum response at the highest velocity tested. The response of neurons classified as band-pass peaked at an intermediate velocity, with the response falling $<$50% of the maximum at the slowest and the fastest velocities tested. Nonselective neurons responded to each stimulus velocity tested with a response $>$50% of the maximum. A similar classification scheme was used for stimulus size tuning.

Histology

At the termination of each recording session, under deep urethan anesthesia, hamsters were perfused via the left ventricle with PBS (0.1 M, pH 7.4) followed by 10% neutral buffered formalin. Brains were removed, postfixed in the same fixative for $\geq$24 h, and stored in PBS containing 30% sucrose for cryopreservation. They were then sectioned coronally at 50 μm and mounted for Nissl staining with cresylecht violet (Chroma Gesellschaft, Münster, Germany).

RESULTS

We have measured the size and velocity tuning of single neurons in the hamster SC to examine the role of NMDA receptors in the normal development and compensatory plasticity of these complex RF properties. NMDA receptors were chronically blocked from birth by implants of D-APV in Elvax, derived from the same batch prepared for our previous study (Huang and Pallas 2001). We estimated D-APV release from the Elvax strip based on scintillation counts. The mean D-APV release was 534 pmol/mm^2 × 48 h prior to implantation and 98 pmol/mm^2 ×48 h on the day of recording response properties. These values of D-APV were comparable to those seen previously (Huang and Pallas 2001).

To ensure that the population of neurons characterized in this study was comparable to those from our previous study (Huang and Pallas 2001), we determined whether the effects of NMDA receptor blockade on RF size were similar across the two studies. We found that the increase in RF size of single SC neurons in the D-APV and PT/D-APV groups compared with the normal group in this study was comparable to that reported previously (Huang and Pallas 2001). The mean (± SE) rostrocaudal RF diameter of single SC neurons in Normal, D-APV and PT/D-APV animals was 9.32 ± 0.34, 13.5 ± 0.44, and 19.5 ± 1.3°, respectively (Fig. 1*A*). The mean RF size of neurons in each group was significantly different from the other two groups (1-way ANOVA, $P < 0.001$). As seen in the previous study, APV treatment shifts the entire population to larger RF diameters (Fig. 1*B*). These results confirm that velocity and size tuning of SC neurons in the experimental animals were created under conditions of increased afferent/target convergence ratios and therefore allowed us to determine the effects of increased convergence on these response properties.

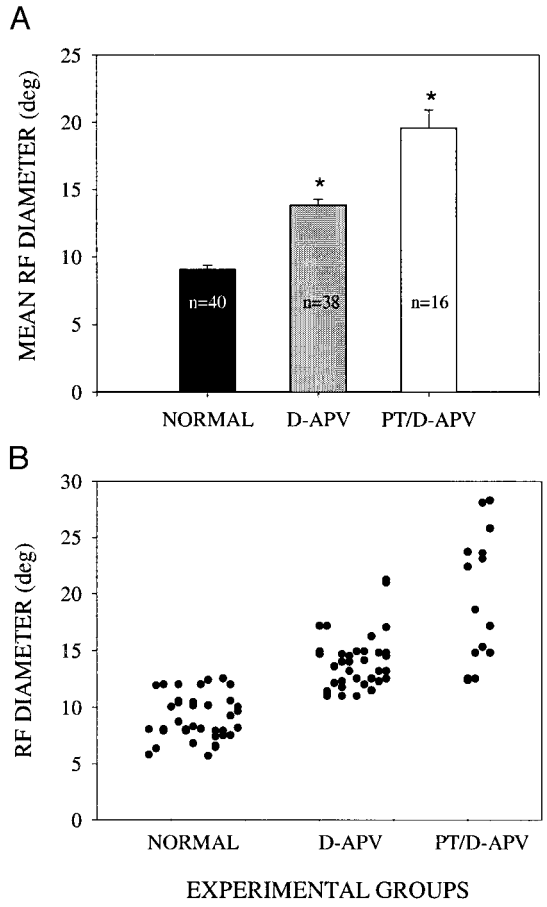

FIG. 1. Receptive field (RF) diameters are significantly larger in experimental groups with *N*-methyl-D-aspartate (NMDA) receptor blockade. *A*: histogram of RF diameters of single superior colliculus (SC) neurons for each group of animals (bars indicate means ± SE). *, significant differences ($P < 0.001$) as compared with the normal group. The number of cells in each group from which mean RF diameters were calculated is indicated within each bar. The mean RF diameter of the D-2-amino-5-phosphonovaleric acid (D-APV) groups was larger than that of the normal group, confirming that NMDA receptors contribute to the refinement of RF diameters during normal development. The RF diameters in the PT/D-APV group were in turn significantly larger than those in the D-APV group, confirming that NMDA receptors contribute to the compensation for increased afferent/target ratios following neonatal partial SC lesion. *B*: raw data showing RF diameters for the entire data set. Note that the drug treatment shifts RF size in the entire population. These data establish that the neuronal population in this study resembles that from our prior study (Huang and Pallas 2001).

SC neurons fall into three categories of velocity-selectivity profiles regardless of rearing conditions

Retinorecipient SC neurons in the superficial gray layer generally exhibit velocity tuning (Chalupa and Rhoades 1977; Tiao and Blakemore 1976). In single-unit recordings from the SC of normal animals, consistent with previous studies, we found that most neurons preferred slow-moving stimuli (low-pass velocity tuning), but a small proportion of neurons exhibited high-pass or band-pass velocity tuning. We hypothesized that if velocity tuning results from spatiotemporal convergence of retinal afferents, then the increased convergence of afferents in the two experimental groups should affect the velocity tuning of retinorecipient SC neurons. To test this hypothesis, we recorded the responses from single SC neurons while presenting stimuli moving across the RF at varying velocities from 5 to 45°/s. We found that chronic NMDA receptor blockade during both normal and postlesion development did not alter the categorical distribution into three velocity tuning categories. Figure 2 shows how these categories were defined by presenting examples of single units from the three categories of velocity tuning profiles under the different rearing conditions. Low-pass neurons such as those shown in Fig. 2*A* responded best to stimuli moving <15°/s, and their response level decreased gradually with increasing velocities. Of 45 neurons recorded in normal SC, the vast majority (42 cells or 93.3%) exhibited a similar low-pass velocity-selectivity profile (Fig. 2*D*). High-pass velocity-selective cells, such as the examples in Fig. 2*B*, were found rarely (1 cell or 2.2%) and responded best to faster stimuli (>25°/s), with responses falling <50% of maximum at lower velocities but >50% of maximum at the highest velocity tested. Neurons such as those shown in Fig. 2*C* (2 cells or 4.4%) were broadly defined as band-pass because they responded better to a stimulus moving at an intermediate velocity with responses falling <50% of maximum at the highest and lowest velocities tested. Band-pass and high-pass neurons are rare, and together comprised only 6.7% of the population in the SC of normal hamsters (Fig. 2*D*). None of the SC cells recorded in this study could be classified as nonselective for stimulus velocity; they were all velocity selective based on our 50% criterion.

A wide range of best velocities was found in all three experimental groups, but due to the rarity of high- and band-

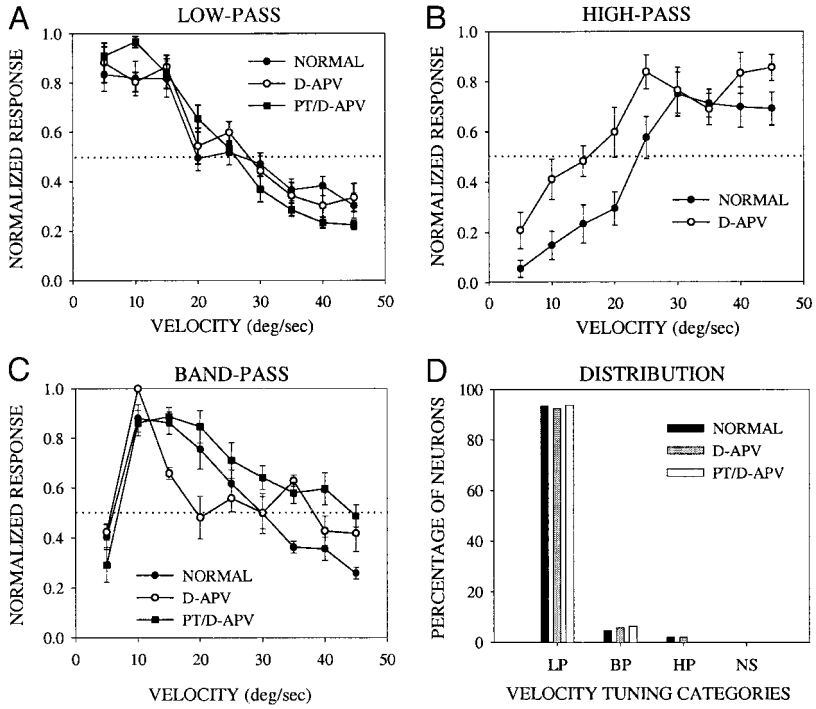

FIG. 2. NMDA receptor blockade does not alter the categorical distribution of velocity tuning. A 50% response criterion (· · · in *A–C*) was used to classify neurons into different categories of velocity preference. *A*: low-pass neurons in all 3 groups of animals responded best to slowly moving stimuli with responses decreasing <50% of the maximum at the highest velocity tested. *B*: high-pass neurons responded best at the highest velocities tested with poor responses at low velocities. No high-pass neurons were found in the PT/D-APV group. *C*: band-pass cells preferred stimuli of intermediate velocities and responded at <50% of the maximum at the highest and lowest velocities. *D*: distribution of the various types of velocity tuning across the 3 experimental groups. In the normal group, the vast majority of cells were low-pass tuned. High- and band-pass cells were rarely encountered. The distribution was not significantly altered after NMDA receptor blockade, suggesting that NMDA receptors are not specifically involved in sculpting a particular category of velocity tuning profile.

pass neurons, sufficient data were not available to perform a population comparison of average best velocities. However, a comparison across the three groups of animals showed that the categories of velocity-selectivity profiles in the D-APV and PT/D-APV groups were comparable to those seen in normal animals (Fig. 2*D*; χ^2 test, $\chi^2 = 2.34$ df. $= 4$, $P > 0.6$). These results indicate that NMDA receptor blockade and the resulting increase in RF sizes did not alter the distribution of neurons into the three velocity tuning categories in the SC. This suggests that NMDA receptors are not specifically involved in establishing categories of velocity tuning during normal development. Moreover, activity mediated by the NMDA receptors does not appear necessary for postlesion maintenance of the categorical distribution of velocity tuning.

Chronic NMDA receptor blockade does not affect velocity tuning in either intact or partially lesioned SC

We also examined two other aspects of velocity tuning: best velocity of individual SC neurons and velocity tuning of the entire population of SC neurons that were recorded. Consistent with the results on velocity tuning categories, single-unit recordings in adult hamsters showed that mean best velocities in the D-APV (6.37 ± 0.44°/s) and PT/D-PV (6.58 ± 0.53°/s) groups were not significantly different from each other or from the mean best velocity in the normal (5.83 ± 0.32°/s) group (*t*-test, normal vs. D-APV, $P > 0.3$; normal vs. PT/D-APV, $P > 0.2$; D-APV vs. PT/D-APV, $P > 0.7$; Fig. 3A). Thus chronic NMDA receptor blockade during postnatal development did not alter the distribution of best velocity across the population of SC neurons.

To test whether NMDA receptor blockade had any effect on velocity tuning profiles across the population of SC neurons, the responses of the low-pass neurons were normalized and compared across the three groups of animals. The low-pass neurons alone were analyzed in this way because they formed the largest class of tuning profiles and because it was of interest to use a uniform population to compare responses to moving stimuli. No effect of NMDA receptor blockade was observed in low-pass neurons from either intact or lesioned SC (ANOVA, Tukey test for all pairwise comparisons, $P > 0.05$ in all cases; Fig. 3*B*). These results indicated that NMDA receptors are unlikely to be involved in the development of velocity selectivity in normal SC. Moreover, blockade of NMDA receptor activity did not appear to have a detrimental effect on the compensatory maintenance of velocity tuning after neonatal partial lesion of the SC. Thus increasing the convergence of afferent inputs on SC cells, and thereby significantly increasing RF size in both experimental groups, had no effect on stimulus velocity tuning in the SC.

SC neurons fall into four categories of stimulus size tuning selectivity profiles regardless of rearing conditions

SC neurons, unlike neurons in the retinogeniculostriate pathway, exhibit characteristic tuning to stimuli much smaller than the classical RF. We hypothesized that if this size tuning results from spatiotemporal summation across multiple retinal afferents, then the experimentally induced increase in afferent convergence should alter the size tuning profiles. To test this, we recorded the responses of single SC cells in response to stimuli of varying sizes. We found that NMDA receptor blockade during either normal or postlesion development does not alter the three-category distribution of stimulus size tuning. Figure 4 shows representative examples of stimulus size tuning profiles from single units recorded in the SC of normal, D-APV, and PT/D-APV hamsters. Neurons such as those shown in Fig. 4*A* were classified as low-pass because they responded best to a light spot with a diameter of 2.5°, and their responses decreased gradually with increasing stimulus diameter. Neurons like those shown in Fig. 4*B* responded best to the largest stimulus sizes tested in this study and were classified as high-

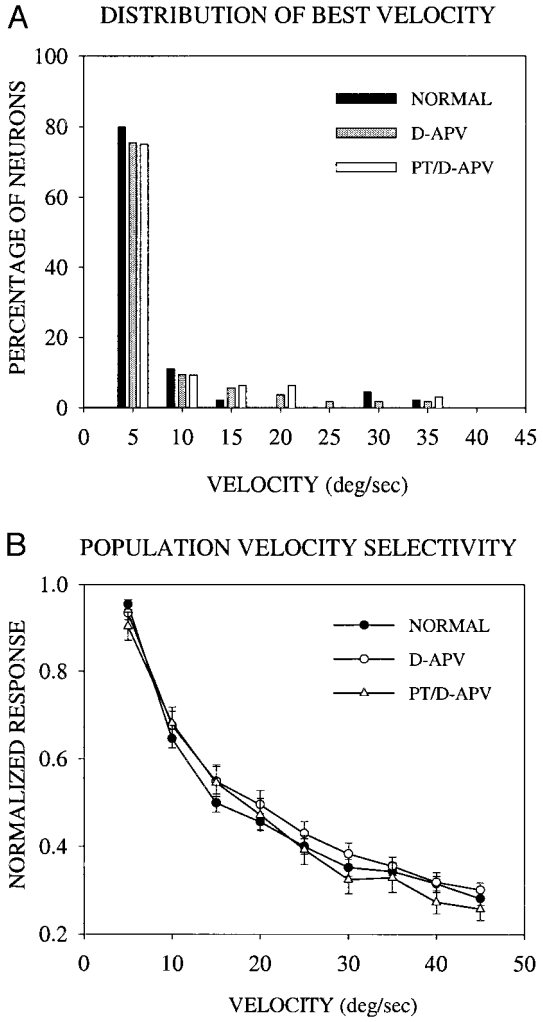

FIG. 3. NMDA receptor blockade does not alter mean best velocities or population velocity selectivity. *A*: the mean best velocity of the SC neurons in the D-APV and the PT/D-APV groups was not statistically different from the mean best velocity in the normal group. In all 3 groups, the slowest moving stimulus tested elicited the best response. *B*: among SC neurons in the low-pass category, the velocity tuning curve of the population was not affected by the drug treatment. These results suggest that NMDA receptors do not play a role in the normal developmental sculpting of velocity tuning, and that they do not contribute to the compensatory maintenance of velocity tuning after early partial SC lesion.

pass. Neurons like the examples shown in Fig. 4*C* were classified as band-pass because of their strong response to intermediate stimulus sizes and weak response to smaller and larger stimuli. As with velocity tuning, the band-pass category for stimulus size was broadly defined, with selectivity for any intermediate stimulus size considered as an example of band-pass tuning. Neurons such as those shown in Fig. 4*D* were classified as nonselective because their responses did not fall <50% of maximum for any stimulus tested. We did not observe any trend for the experimental treatments to affect the distribution of band- or high-pass neurons between groups. Of 39 neurons recorded in normal SCs, 24 (61.5%) were low-pass, 4 (10.3%) were band-pass, 7 (17.9%) were high-pass, and 4 (10.3%) were nonselective. A statistical comparison of the categories of stimulus size selectivity profiles across the three groups of animals showed that, as with velocity tuning, SC neurons were proportioned into the same classes of stimulus size selectivity regardless of whether they were reared with NMDA receptor blockade, in the context of either an intact or a partially lesioned SC (Fig. 4*E;* χ^2 test, $\chi^2 = 4.87$, df $= 6$, $P > 0.5$). Because the distribution of neurons into categories of stimulus size tuning remained unaltered, these results indicate that NMDA receptors are unlikely to be involved in the generation of any particular size tuning category, either during normal development or following partial SC lesion.

Chronic NMDA receptor blockade does not affect stimulus size tuning in either intact or partially lesioned SC

The distributions of best stimulus size and the SC neuronal population size tuning profiles were examined as outlined in the preceding text for velocity tuning. On quantitative analysis of the population of single units from each group, it was found that chronic NMDA receptor blockade had no effect on the distribution of best stimulus size (Fig. 5*A*) in either intact or compressed maps. The best stimulus size distributions in the D-APV (6.28 ± 0.67°) and PT/D-APV (5.01 ± 0.72°) groups were not significantly different from each other or from the best stimulus size distribution in normal SC neurons (5.21 ± 0.74°; *t*-test, normal vs. D-APV, $P > 0.2$; normal vs. PT/D-APV, $P > 0.8$; D-APV vs. PT/D-APV, $P > 0.2$).

As with population velocity tuning, only units classified as low-pass were compared for the analysis of the effect of NMDA receptor blockade on stimulus size tuning profiles across the population of SC neurons. A population-wide comparison of stimulus tuning was not performed on the band- or high-pass neurons due to their paucity in the samples. No difference was observed in the shape of tuning curves to stimulus size (Fig. 5*B*), showing that the decrease in response level with increasing stimulus size was similar in all three groups (ANOVA, Tukey test for pairwise comparisons, $P > 0.05$ for all comparisons). Thus as for stimulus velocity selectivity, chronic NMDA receptor blockade in intact or partially lesioned SC had no effect on stimulus size selectivity. These results indicate that NMDA receptors are unlikely to be involved in generating stimulus size selectivity in normal SC or in the compensatory maintenance of stimulus size selectivity following partial SC lesion. These results also show that altering the afferent/target convergence ratio has no effect on stimulus size tuning, suggesting that tuning has an origin independent of spatiotemporal summation of information from retinal inputs.

The results of this study show that NMDA receptor blockade during postnatal development has no effect on selectivity of neurons in superficial SC for stimulus velocity and size in normal hamsters, or on the conservation of these properties after neonatal partial SC lesions. These results taken together show that tuning of single SC neurons to the size and velocity of movement of a visual stimulus occurs independently of NMDA receptors in SC, map compression, afferent/target convergence, or RF size.

DISCUSSION

During normal development of the visual system, the mapping of retinocollicular connections, or indeed any topographic connections, is guided by at least two requirements: maintenance of topographic order and establishment of a correct

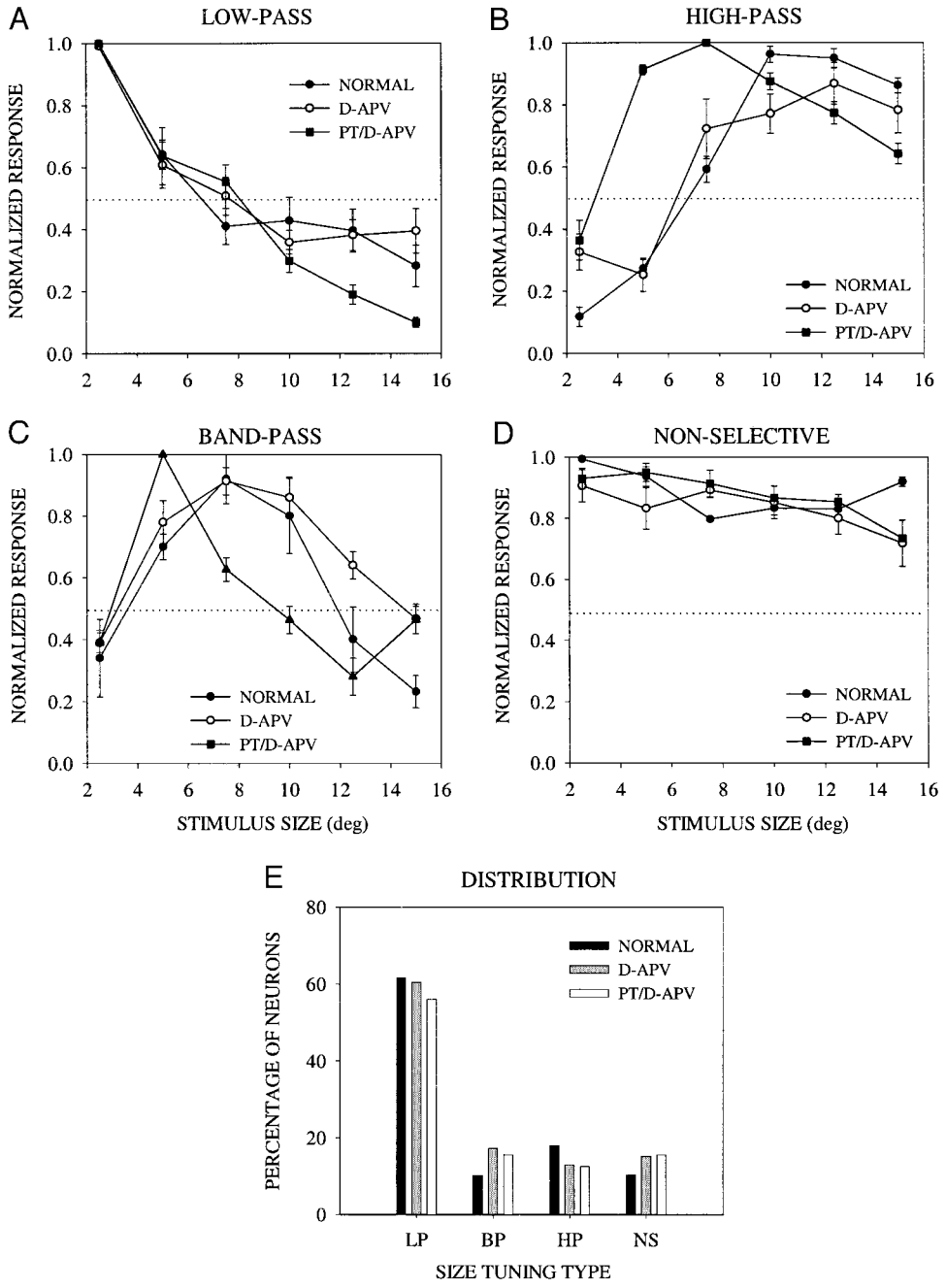

FIG. 4. NMDA receptor blockade does not alter the distribution of neurons into categories of stimulus size tuning. A 50% response criterion (· · · in *A–D*) was used to classify neurons into different categories of stimulus size preference. *A*: low-pass neurons in all 3 groups of animals were defined as those that responded best to small stimuli with responses decreasing <50% of maximum at the highest stimulus size tested. *B*: high-pass neurons responded best to the largest stimuli tested with poor response to the smallest sized stimuli. *C*: band-pass neurons preferred stimuli of intermediate sizes and responded at <50% of maximum to the largest and smallest stimuli. *D*: the response magnitude of nonselective neurons did not change by >50% at any size tested. *E*: nearly 60% of the neurons in all 3 experimental groups exhibited low-pass tuning to stimulus size. The distribution was not significantly altered by NMDA receptor blockade during normal development or after partial collicular lesion.

numerical match between afferents and their target neurons. Adhering to both requirements is likely to be critical in determining normal behavior and perceptual acuity. In the case of retinocollicular connections, after partial lesion of the SC in neonates, the degree to which compensatory reorganization is behaviorally adaptive would depend on the degree to which basic response properties are affected. Previous studies have shown that despite large mismatches between afferent/target convergence ratios, RF size and stimulus tuning properties of individual collicular neurons are conserved (Pallas and Finlay 1989) and that this is based on an NMDA receptor-activity-dependent process (Huang and Pallas 2001). This study evaluated the hypothesis that NMDA receptor-dependent activity is also required for construction of velocity and size tuning circuitry during normal development and for postinjury compensation through its ability to regulate afferent/target convergence. Our main finding is that stimulus velocity and size tuning of SC neurons are not affected by blockade of NMDA receptor activity during postnatal development, despite the resulting mismatch of afferent/target convergence ratios in intact or partially lesioned SC. This suggests that NMDA receptor activity within the SC is not necessary for either construction or refinement of stimulus velocity and size tuning of SC neurons during normal development or after neonatal partial SC ablation. Thus across large variations in map compression and afferent convergence, and despite the possible decrease

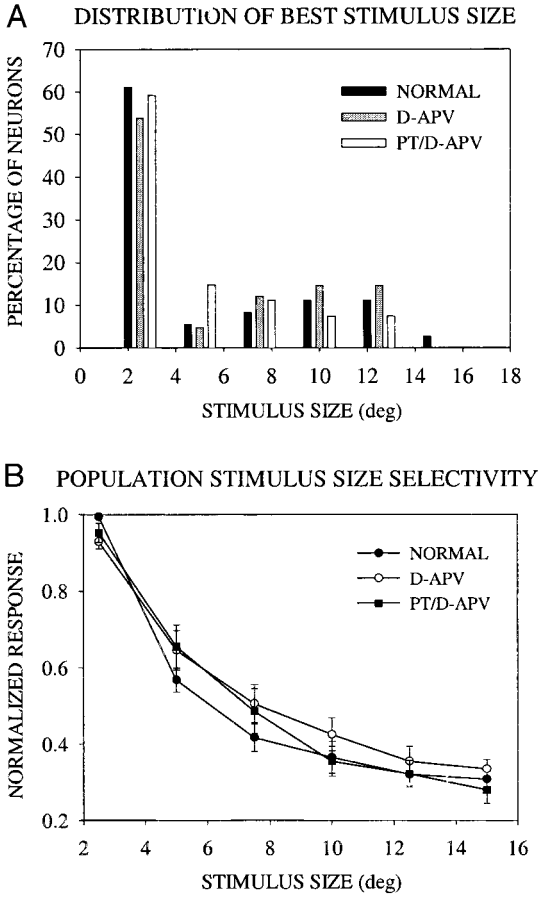

FIG. 5. NMDA receptor blockade does not alter mean best stimulus size or population size selectivity. *A*: The mean best stimulus sizes of the SC neurons in the D-APV and the PT/D-APV groups were not statistically different from the mean best stimulus size of SC neurons in the Normal group. In all three groups, the smaller stimuli elicited the best responses. *B*: In the low-pass category, the rate of decrease of neural responses with increasing stimulus size was also not significantly affected by the drug treatment. These results suggest that NMDA receptors do not play a role in normal developmental sculpting of stimulus size tuning, and do not contribute to the compensatory maintenance of stimulus size tuning following early partial collicular lesion.

in acuity resulting from enlarged RFs after early brain damage, the ability to code multiple aspects of visual stimuli is maintained in SC by NMDA-receptor independent mechanisms.

Implications for neural mechanisms underlying stimulus velocity and size tuning in normal SC

Complex response properties such as stimulus velocity and size tuning could arise from integration of activity converging from afferent structures to the target structure (hierarchical processing), from circuitry intrinsic to the target, from selective projections from afferent structures that are already tuned (parallel processing) or from a combination thereof. These possibilities have given rise to multiple hypotheses for how visual response properties are constructed. For example, one proposed model for velocity selectivity depends on spatiotemporal interactions between excitatory and inhibitory areas of a visual neuron's RF (Barlow and Levick 1965; Goodwin and Henry 1978). This idea takes advantage of the fact that stimulus velocity determines the time interval between the entrance of a moving stimulus into the inhibitory and excitatory subfields of a neuron's RF. For slowly moving stimuli, the transition from inhibitory to excitatory subfields and vice versa takes longer than for fast-moving stimuli. This results in differences in temporal overlap of inhibitory and excitatory synaptic inputs and can result in velocity tuning. The model predicts that increasing the RF diameter of visual neurons would change velocity tuning. Neurons with different excitatory RF sizes are likely to have different dynamics of temporal overlap between excitatory and inhibitory inputs for different velocities, resulting in different velocity tuning. Consistent with this argument, a correlation has been reported between RF size and velocity tuning in visual cortex (Orban et al. 1981), lateral geniculate nucleus (Hess 1979), and SC (Waleszczyk et al. 1999). Typically, neurons with larger RFs respond better to faster stimulus velocities than neurons with smaller RFs. However, we did not find that experimentally induced increases in RF size altered velocity tuning but instead found that velocity tuning was unaffected in all respects in normal and partially lesioned cases. This was true even though in our paradigm the effect of RF size on velocity tuning could be assessed at similar map locations. This shows that RF size of SC neurons is not a good predictor of velocity tuning. The lack of effect of increased RF size on velocity tuning suggests that spatiotemporal integration of afferent excitatory inputs to individual SC neurons does not contribute significantly to velocity tuning of SC neurons.

The results of this study point toward the likelihood that velocity tuning in SC is not an emergent property but reflects tuning already present in the retina imposed on the SC by selective parallel projections. The depth of recording (<100 μm) in this study corresponds to the superficial part of the stratum griseum superficiale (SGS) in the SC (Mooney et al. 1985). Although all types of retinal ganglion cells (RGCs) project to the SC in hamsters, neurons innervated by rapidly conducting or Y-type RGCs are usually located within the deeper layers of the SGS and within the stratum opticum (Fukuda et al. 1978; Mooney et al. 1985). Neurons in the superficial SGS receive input only from the slowly conducting, W-type RGCs (Johnson and King 1982). The W-type RGCs are typically tuned to slow-moving stimuli, and their selective projections to superficial SC may be responsible for the selectivity for slow-moving stimuli that we observed. The lack of effect of altered afferent/target convergence ratios due to NMDA receptor blockade in the intact and partially lesioned SC could be explained if the experimental manipulations resulted in increased input only or mainly from similarly tuned W-type RGCs. However, the possibility that the NMDA receptor blockade alters inhibitory circuitry within the SC to preserve velocity tuning cannot be excluded and is currently under study.

In the hamster SC, close to 60% of neurons prefer stimuli that occupy less than half of the RF (Pallas and Finlay 1989; Stein and Dixon 1979). The fact that the preferred stimulus size and distribution of size tuning profiles did not change after the experimentally induced increases in RF size suggests that the best stimulus size is not a fixed, optimal percentage of the RF size but rather a reflection of interactions between spatial summation and inhibition within the eRF (Stein and Dixon 1979). In cells that were classified as low- or band-pass, neural responses were maximal when an appropriate level of spatial summation was reached. Increasing stimulus size further ap-

parently recruited inhibition and the response began to decrease. The fact that stimulus size tuning did not change with experimentally induced increases in RF size suggests that the thresholds for spatial summation and inhibition are set independently of RF size and that NMDA receptors are not involved in this process.

Conclusions

While NMDA receptors play a role in refinement of RF size in the intact SC and in refinement of RF size and compensation for mismatched afferent/target ratios in the partially lesioned SC, they are apparently not involved in the creation of velocity and size tuning in either developmental scenario. Thus it appears that NMDA receptor-independent mechanisms are responsible for the creation and conservation of response selectivity for attributes of stimulus motion and size in hamster SC neurons. This independence may facilitate preservation of visuomotor function that depends on stimulus attributes such as size and velocity of behaviorally relevant stimuli, while allowing topographic maps to conform to changing convergence ratios during developmental cell death and collateral elimination. The same mechanisms can act as a substrate to facilitate recovery of function after an injury. Such a lack of conflict between NMDA receptor-activity-dependent and -independent processes in creating multiple RF properties may be of broad applicability in other brain regions and during evolutionary changes in afferent/target population matching.

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