Development/Plasticity/Repair

Inhibitory Plasticity Facilitates Recovery of Stimulus Velocity Tuning in the Superior Colliculus after Chronic NMDA Receptor Blockade

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The developing nervous system is shaped in important ways by spontaneous and stimulus-driven neural activity. Perturbation of normal activity patterns can profoundly affect the development of some neural response properties, whereas others are preserved through mechanisms that either compensate for or are unaffected by the perturbation. Most studies have examined the role of excitation in activity-dependent plasticity of response properties. Here, we examine the role of inhibition within the context of response selectivity for moving stimuli. The spatial extent of retinal input to the developing hamster superior colliculus (SC) can be experimentally increased by chronic NMDA receptor (NMDAR) blockade. Remarkably, stimulus velocity tuning is intact despite the increase in excitatory inputs. The goal of this study was to investigate whether plasticity in surround inhibition might provide the mechanism underlying this preservation of velocity tuning. Surround inhibition shapes velocity tuning in the majority of superficial layer SC neurons in normal hamsters. We show that despite the NMDAR blockade-induced increase in feedforward excitatory convergence from the retina, stimulus velocity tuning in the SC is maintained via compensatory plasticity in surround inhibition. The inhibitory surround increased in strength and spatial extent, and surround inhibition made a larger contribution to velocity tuning in the SC after chronic NMDAR blockade. These results show that inhibitory plasticity can preserve the balance between excitation and inhibition that is necessary to preserve response properties after developmental manipulations of neural activity. Understanding these compensatory mechanisms may permit their use to facilitate recovery from trauma or sensory deprivation.

Key words: **traumatic brain injury; rodent; retinotectal; homeostatic plasticity; inhibitory plasticity; visual development**

Introduction

The relative contribution of activity-dependent and -independent processes to the development of neural response properties is a topic of fundamental importance. Given that both excitatory and inhibitory activity levels vary during development, neural response properties may become independent of activity fluctuations through homeostatic processes that compensate for changes in activity levels (Turrigiano et al., 1998; Varela et al., 1999; Turrigiano and Nelson, 2004). Indeed, it has been observed that experimental manipulations of early visual experience can have little effect on some response properties and major effects on others (Rhoades and Chalupa, 1978a; Sengpiel et al., 1998; Fagiolini et al., 2003; Razak and Pallas, 2006). It remains unknown in many cases whether a lack of effect of altered activity on response properties is caused by activity independence of the underlying mechanisms or by compensatory processes that operate to maintain the response property. Here, we present evidence for the latter mechanism in the superior colliculus (SC) of the hamster.

We have shown previously that blocking NMDA receptors (NMDARs) in the SC throughout postnatal development increases receptive field (RF) size (Finlay et al., 1979; Huang and Pallas, 2001). However, stimulus size and velocity tuning are unaffected (Razak et al., 2003), suggesting that their preservation occurs through a non-NMDAR-dependent mechanism. In this study, we examined the possibility that changes in inhibitory circuitry might underlie the preservation of velocity tuning under NMDAR blockade. Changes in inhibitory input can compensate for changes in excitatory input induced by manipulations of neural activity in single cells during development (Turrigiano, 1999), and there is growing evidence that homeostatic inhibitory plasticity is also present at the network level (for review, see Pallas et al., 2006).

In hamsters, velocity tuning in the majority of superficial SC neurons is shaped by surround inhibition (Razak and Pallas, 2005). The timing between excitatory and inhibitory inputs to a neuron generated by stimulus movement is critical for velocity tuning in SC (Razak and Pallas, 2005), as it is in visual cortex (Goodwin and Henry, 1978; Patel and Sillito, 1978). Increasing the size of the excitatory RF would alter the relative timing of excitatory and inhibitory inputs and therefore would be expected

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to affect velocity tuning. Given the role of inhibition in velocity tuning, we hypothesized that the conservation of velocity selectivity seen after experimental expansion of the excitatory RF results from a compensatory change in the inhibitory surround.

To test this hypothesis, we recorded from SC neurons in normal hamsters and in hamsters reared with chronic NMDAR blockade of SC, comparing the strength and size of surround inhibition and the contribution of surround inhibition to velocity tuning. We present evidence that chronic NMDAR blockade causes an increase in the strength and size of surround inhibition. We show that this increased strength of surround inhibition is important for maintaining velocity tuning in SC neurons with enlarged RFs. Such changes could preserve a homeostatic balance between inhibition and excitation, either during normal developmental or evolutionary changes in excitatory convergence, or in compensation for perinatal trauma.

Materials and Methods

Animals. Syrian hamsters (*Mesocricetus auratus*), bred in the Georgia State University (GSU) animal facility with breeding stock purchased from Charles River Laboratories (Wilmington, MA), were used in this study. All procedures used on animals were reviewed and approved by the GSU Animal Care and Use Committee and were consistent with National Institutes of Health and Society for Neuroscience guidelines.

Experimental design. This study sought to determine surround inhibition and its contribution to velocity tuning in the SC after chronic blockade of NMDARs. NMDARs were blocked chronically using the antagonist D-2-amino-5-phosphonovaleric acid (D-APV) as previously described in detail (Huang and Pallas, 2001; Razak et al., 2003). Briefly, the D-APV group of animals had the biologically active form of the NMDAR antagonist D-APV (Tocris Neuramin, Langford, UK) in Elvax polymer (DuPont, Stevenage, UK) implanted over the SC on the day of birth and throughout postnatal development. This treatment results in an expansion of single-unit receptive fields in the SC (Huang and Pallas, 2001; Razak et al., 2003). We have shown previously that the inactive form of APV (L-APV) has no effect on response properties in hamster SC, when either acutely or chronically applied (Huang and Pallas, 2001). Because we were interested in the effects subsequent to an increase in RF diameter, an L-APV group was not included in this study. After rearing the hamsters to adulthood (>70 d postnatal) under pharmacological blockade of NMDARs, surround inhibition and its contribution to velocity tuning were assessed using *in vivo* extracellular single-unit recording.

Elvax preparation. The D-APV-impregnated Elvax polymer [generously donated by Dr. Adam Smith (University of Oxford, Oxford, UK)] was prepared according to published methods (Silberstein and Daniel, 1982; Schnupp et al., 1995; Smith et al., 1995). The polymer contained 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 obtained through scintillation counts before and after implantation. The initial procedures before implantation and the drug release characteristics of the polymer have been reported previously (Smith et al., 1995; Huang and Pallas, 2001). Briefly, 100- or 200-μm-thick Elvax sheets were preincubated for 48 h in PBS, pH 7.4 (0.5 ml), to prevent exposing the SC to an initial burst of drug release on rehydration of the polymer. The release rate is equivalent for the two different thicknesses used (Smith et al., 1995). The Elvax was then inserted under the skull and over the SC in the experimental animals. After implantation on the surface of the SC, the polymer continues to release the drug at a gradually declining rate for ~12 months (Smith et al., 1995; Huang and Pallas, 2001). We have demonstrated that this Elvax preparation successfully blocks a substantial proportion of the NMDA receptor-dependent glutamate component of retinocollicular transmission, without reducing either the AMPA receptor-dependent component or visual transmission (Huang and Pallas, 2001). The same batch of D-APV in Elvax was used in both studies. The estimated mean D-APV release based on scintillation counts was 534 pmol/mm^2 × 48 h before implantation and 98 pmol/mm^2 × 48 h on the day of recording response properties. These values were comparable with those reported in Huang and Pallas (2001).

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 via a face mask. 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 dura and over the right SC. The pups were returned to maternal care after closure of the wound and recovery from anesthesia.

Electrophysiological recordings were obtained from adult hamsters after anesthetization with urethane (0.7 g/ml, 0.03 ml/kg, i.p. in 3–4 aliquots spaced at 20 min intervals), a drug that affects various neurotransmitter systems fairly equally (Maggi and Meli, 1986; Hara and Harris, 2002; Sceniak and Maciver, 2006) and provides long-lasting, deep anesthesia. The hamsters used in this study were between 70 and 300 d of age. The Elvax sheet was therefore present on the SC for 70–300 d. Scintillation counts show that the release of D-APV is typically high during the first few 2 d and then tapers off to a steady but low release rate after that (Smith et al., 1995; Huang and Pallas, 2001). Therefore, regardless of the age of electrophysiological recording, the strongest D-APV exposure would have occurred before 1 month of age. The effects of NMDAR blockade were similar for the younger and older animals (see Results below). Pupils were dilated and accommodation was paralyzed 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 urethane given as warranted. After performing a craniotomy over the SC, the visual cortex was aspirated to facilitate viewing the surface of the SC for electrode placement and the removal of the Elvax polymer in D-APV animals. It is not possible to perform electrophysiology in the SC of the D-APV group without removing the Elvax strip that sits between the cortex and the SC. The occipital cortex has to be removed to access the Elvax strip. The visual cortex was thus removed in its entirety in each experiment; the remaining cortex does not respond to visual stimulation. Therefore, there is unlikely to be variability in the extent of visual cortex removed.

It is unlikely that the acute removal of visual cortex has a major influence on the interpretations of this study for four reasons. First, both groups of animals that are compared in this study had the visual cortex acutely removed. Second, Fortin et al. (1999) and Rhoades and Chalupa (1978b) have shown that the influence of cortical connections on SC response properties in rodents is minimal. The effect is present for direction selectivity but not for velocity selectivity. Third, previous studies have also concluded that SC surround inhibition is not affected by acute removal of visual cortex (Wickelgren and Sterling, 1969). Fourth, velocity tuning in normal hamster SC is almost identical to decorticate hamster SC [compare Stein and Dixon (1979) and Razak et al. (2003)].

To maintain eye position without paralyzing the animal during the recording session, the conjunctivum was stabilized with 6-0 silk suture. The optic disk was replotted after every electrode pass to confirm that eye position remained stable, which in hamsters is typically not a concern (Pallas and Finlay, 1989). The eye was covered with a fitted plano contact lens for protection during the recording session, and the brain was kept covered with sterile saline.

Visual stimulation and electrophysiological recording. All of the single units recorded in this study had their RFs centered within ±15° of the optic disk and were isolated within 200 μm of the SC surface, consistent with previous studies (Huang and Pallas, 2001; Razak et al., 2003). Insulated tungsten microelectrodes (1–3 MΩ; FHC, Bowdoinham, ME) were lowered perpendicular to the surface of the SC to isolate visually responsive cells in the retino-recipient superficial gray layer of the right SC. The approximate location of the excitatory receptive field (eRF) and its preferred stimulus velocity were determined, and a 14 inch computer monitor was moved to the location of the eRF at a distance of 40 cm from the left eye. A Sergeant Pepper graphics board (Number Nine, Cambridge, MA) was used in conjunction with STIM software to generate visual stimuli. Data were acquired by CED 1401 hardware and processed by Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Superior colliculus neurons of rodents prefer small, slowly moving

spots (Tiao and Blakemore, 1976; Rhoades and Chalupa, 1978a; Stein and Dixon, 1979; Pallas and Finlay, 1989; Razak et al., 2003) and respond poorly to gratings or rapidly moving stimuli (>45°/s). The eRF diameter of each neuron was determined by sweeping a single spot of light (1° diameter) from the top to the bottom of the computer monitor screen. Successive sweeps started 2° lateral to the previous sweep, allowing a determination of the nasotemporal extent of the eRF. The light spot was swept at either 5°/s or 30°/s velocity, depending on whether the neuron responded better to slowly or rapidly moving stimuli. The estimated RF size did not change with the velocity of the stimulus used.

To determine the extent of the inhibitory surround, two spots of light (each 1° in diameter) were swept simultaneously from the top to the bottom of the monitor screen (Razak and Pallas, 2005). During each successive sweep, the second spot of light started 2.6° farther away from the previous location, whereas the first spot was swept through the center of the eRF. This allowed us to determine the spatial extent and strength of inhibition of the response to the first spot caused by the second spot. The time interval between each sweep was set at 10 s to avoid adaptation. Each stimulus pair was repeated three to seven times.

Determination of velocity selectivity. The method for determining velocity tuning in the hamster SC was described in detail previously (Razak and Pallas, 2005). Briefly, neural selectivity for stimulus velocity was determined by sweeping a 2.5° diameter spot of light at 5–45°/s increasing at 5°/s increments. For most of the experiments in this study, the direction of stimulus sweep was temporal-to-nasal with respect to the animal. The choice of stimulus velocities used was guided by previous results showing that the majority of hamster SC neurons are selective for slowly moving (≤10°/s) stimuli (Tiao and Blakemore, 1976; Chalupa and Rhoades, 1977; Stein and Dixon, 1979; Pallas and Finlay, 1989; Razak et al., 2003). Each stimulus set was typically repeated at least five times, although fewer trials were collected in some cases in which a large number of tests were being done on the neuron. In all cases, the responses were quite consistent, as reflected in the small error bars when between-trial comparisons were made.

Neurons were categorized as exhibiting low-pass (LP), bandpass (BP), or high-pass (HP) tuning to stimulus velocity (Razak et al., 2003). A neuron was defined as LP if its response to a stimulus moving between 5 and 15°/s was at least twice that of the least-preferred stimulus. HP neurons were those that responded best to stimuli moving faster than 25°/s and that responded at <50% of their maximum to the lowest velocity tested. Neurons in the BP category responded best to an intermediate velocity, with responses falling below 50% of their maximum at the lowest and at the highest velocities tested. Low-pass neurons generally did not respond better to stationary than moving stimuli, but the response of HP neurons would be expected to decline with stimuli faster than 45°/s based on previous work (Chalupa and Rhoades, 1977; Razak et al., 2003). Nonselective neurons by definition responded at >50% of maximum at all velocities tested.

Determination of the contribution of surround inhibition to velocity tuning. To determine whether suppression from the inhibitory surround contributes to stimulus velocity tuning in the two experimental groups, an opaque barrier was used to mask various parts of the surround. The terms temporal surround (TS) and nasal surround (NS) are used to describe the surround locations relative to the visual field of the animal. TS refers to surround locations on the caudal side of the RF, whereas NS refers to the surround locations on the rostral side of the RF. The mask was positioned to cover the TS or NS up to the edge of the eRF. Velocity tuning was then determined as above with either the NS or the TS masked. Velocity tuning was determined again after removal of the masks to ensure that the effects of masking were temporary and attributable to the masks alone.

Data analysis. To measure neuronal selectivity for velocity, stimulus-driven spikes were counted. Levels of spontaneous activity in superficial SC neurons in anesthetized adult hamsters are very low (Huang and Pallas, 2001). This, coupled with the long interstimulus intervals used in this study, made it possible to use the number of stimulus-evoked action potentials at each velocity to obtain an accurate measure of response selectivity (for details, see Razak and Pallas, 2005).

To determine whether physical masking of the inhibitory surround had any effect on velocity tuning in the SC, responses at each velocity in the control and masked conditions were statistically compared using a two-way ANOVA, with a Tukey test for *post hoc*, pairwise comparisons. For comparison of properties across the recorded population of neurons, regardless of the response magnitude in any particular neuron, normalized curves were constructed by dividing the response at each velocity by the response to the preferred velocity. To determine whether surround inhibition contributes more to velocity tuning in the D-APV group than in the normal group, the increase in response to all velocities after masking was compared between the two populations. In all of the figures, variability of responses across stimulus repetitions is depicted as the SEM.

Results

Effects of chronic NMDAR blockade on surround inhibition

To test the hypothesis that the strength of surround inhibition is increased in SC neurons with larger eRF diameters, we mapped surround inhibition of SC neurons in the D-APV group (60 neurons from 16 hamsters). These data were compared with previously published data from normal hamsters (Razak and Pallas, 2005). Except for the NMDAR blockade, the methods used in the present study were identical to those used on normal hamsters in that previous study.

As published previously for the normal group (Razak and Pallas, 2005), D-APV group neurons could be classified based on the location and strength of surround inhibition; present on only one side of the eRF, present on both sides of the eRF, or weak inhibition on both sides of the eRF. The neuron shown in Figure 1*A* exhibited inhibition on only one side of the RF. This neuron exhibited weak inhibition on the temporal side (TS) of the receptive field. The response of the neuron to the light spot in the center of the RF was not strongly influenced by a second spot of light on the temporal side. On the nasal side (NS), however, the response to the center spot of light was strongly inhibited by the presence of a second stimulus, with maximum inhibition observed between 5 and 13° away from the center of the RF. The example shown in Figure 1*B* showed inhibition on both sides of the RF. The inhibition did not meet our 30% inhibition criterion (see Materials and Methods) on the TS side, however, so this neuron was classified as inhibited on one side of the RF. As seen with the normal group, most neurons (9 of 12) with inhibition on only one side of the RF exhibited stronger inhibition on the NS than the TS.

The neurons shown in Figure 1*C* exhibited >30% inhibition on both sides of the RF with no recovery to control response observable at locations tested in this study. The neuron in Figure 1*D* exhibited a narrow region of surround inhibition. Both neurons were classified as showing inhibition on both sides of the RF. The neurons shown in Figure 1, *E* and *F*, did not show >30% inhibition on either side and were classified as exhibiting weak surround inhibition. Together, these examples show that surround inhibition is present in the SC after chronic blockade of NMDAR, and neuronal types fall into similar categories with respect to their surround inhibition as in the normal group.

Chronic NMDAR blockade, however, increased the percentage of neurons that exhibited surround inhibition (χ^2 test; $p < 0.05$). In the D-APV group of animals, only 7 of 47 (15%) neurons tested on both sides of the eRF exhibited weak surround, compared with 35% in the normal group (Fig. 2*A*). A significantly higher percentage of neurons in the D-APV group than in the normal group exhibited inhibition on both sides of the eRF. The percentage of neurons with inhibition on only one side of the RF was similar in both groups. Across the D-APV population, surround inhibition was stronger at every location except within 5.2°

of the eRF center in the D-APV group compared with the normal group (Fig. 2*B*) (two-way ANOVA, Tukey test for *post hoc*, pairwise comparisons of similar locations in the two groups; $^*p < 0.05$). Because the RFs are enlarged in these animals, the 5.2° location is inside the RF for the D-APV group but not for the normal group. Together, these results show that chronic NMDAR blockade results in increases in the strength and extent of surround inhibition in superficial SC neurons.

The size of the inhibitory surround was correlated with the size of the eRF in the D-APV group. Because the spatial extent of the surround inhibition was broader than could be tested in many neurons (given the monitor size), we used the strength of inhibition at the location farthest from the center of the RF (18.5°) as an indicator of surround size. The strength of inhibition is the reciprocal of the normalized response to the dual stimuli at 18.5°. Figure 3*A–C* shows the relationship between strength of inhibition at 18.5° and the size of the eRF in three individual animals from which at least five neurons were tested. The data shown in Figure 3*A* were from one of the youngest animals (72 d of age) recorded from, whereas the data in Figure 3, *B* and *C*, were from animals older than 6 months. In each of these three hamsters, the strength of inhibition was stronger for neurons with larger eRFs. Figure 3*D* shows the population data from all D-APV neurons from which eRF and surround inhibition were determined. Overall, the increasing strength of inhibition with larger eRF diameter was significant. These data suggest that after an NMDAR blockade-induced increase in eRF diameter, there is a concomitant increase in the extent of the inhibitory surround.

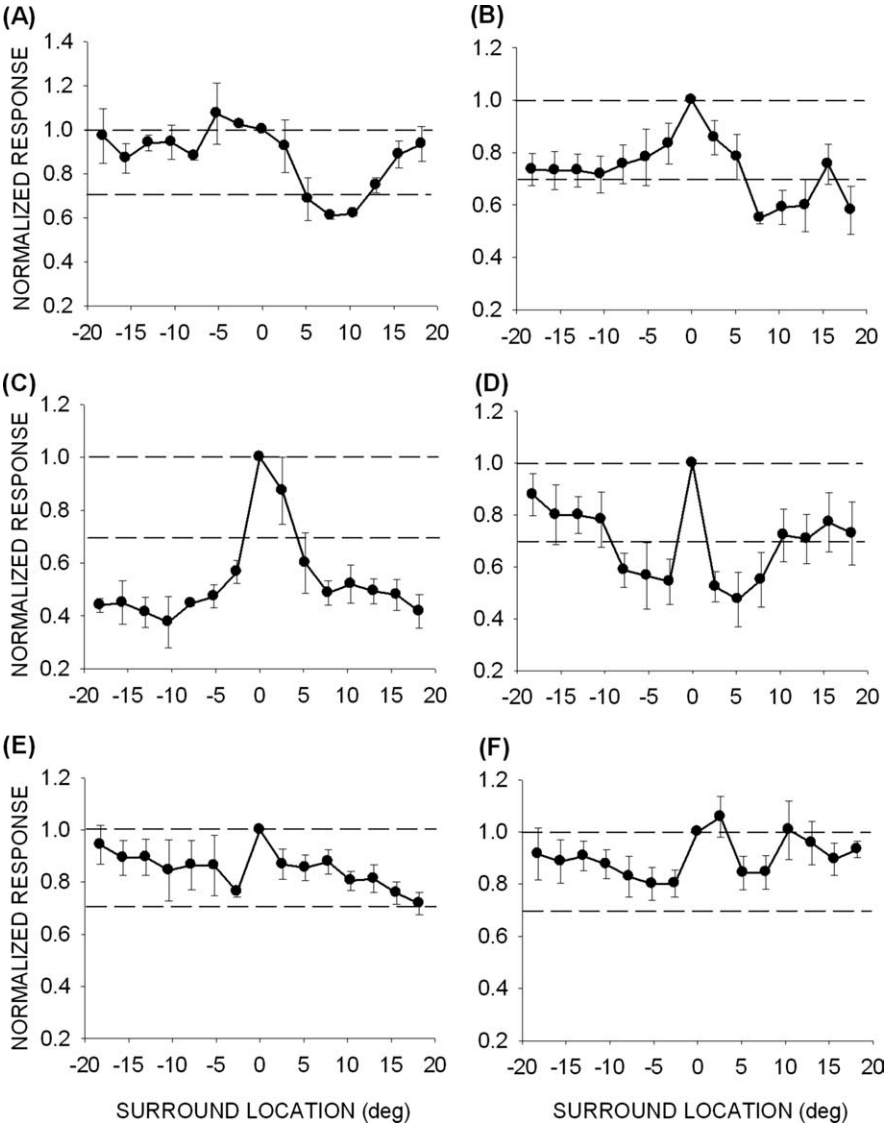

Figure 1. Spatial geometry of surround inhibition in neurons from the D-APV group. The population of SC neurons in the D-APV group exhibited variations in RF arrangement similar to that seen in normal animals. ***A***, ***B***, Neurons in which strong inhibition was present on only one side of the eRF. ***C***, ***D***, Examples of neurons with inhibition on both sides of the eRF. ***E***, ***F***, Neurons with weak surround inhibition.

Consequences of increased surround inhibition on velocity tuning

To test the hypothesis that the contribution of inhibition to velocity tuning was larger in SC neurons with increased eRF diameter, we compared velocity tuning of SC neurons in the D-APV group in the masked and unmasked condition (39 neurons from 13 hamsters). In normal hamsters, the timing of stimulus movement through the excitatory and inhibitory portions of the RF is critical for velocity tuning. Selectivity for stimuli moving slowly (LP) in a temporal to nasal direction arises as a result of inhibition from the NS (Razak and Pallas, 2005). LP neurons respond best when stimuli spend considerable time in the excitatory portion before entering the subsequent inhibitory portion of the RF (NS for temporonasally moving stimuli). Masking the NS increases responses to the nonpreferred, rapidly moving stimuli. Masking the TS has no effect. That is, inhibition from the NS has an effect on LP velocity tuning through backward masking. High velocity-tuned (HP) neurons are suppressed when stimuli linger in the initial inhibitory portion (TS for temporonasally moving stimuli) before entering the excitatory portion of the RF. Masking the TS increases the response to slow-moving stimuli, thereby reducing velocity tuning. Masking the NS has no effect. Therefore, inhibition from the TS has an effect on HP velocity selectivity through forward masking. Chronic NMDAR blockade increases the diameter of the eRF in hamster SC (Huang and Pallas, 2001; Razak et al., 2003). This increase in diameter will alter the timing between inhibition and excitation and thus would be expected to influence velocity tuning. Surprisingly, chronic NMDAR blockade has no effect on velocity tuning (Razak et al., 2003). The finding that chronic NMDAR blockade also increases the strength and extent of surround inhibition led us to hypothesize that the increase in inhibition might compensate for changes in the eRF. This could provide a mechanism for the maintenance of velocity tuning in D-APV-treated animals. If inhibition makes a larger contribution to velocity tuning in the D-APV group, then the increase in responsiveness to nonoptimal velocities caused by surround masking should be higher in the D-APV group than the

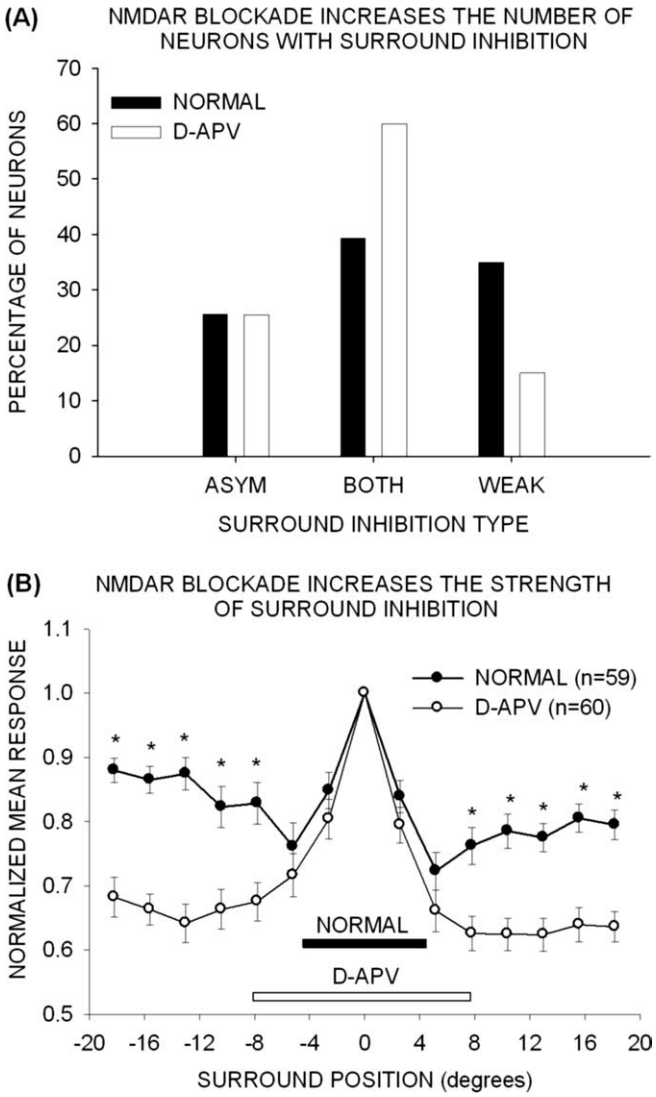

Figure 2. Population summary of the effect of chronic NMDAR blockade on surround inhibition. ***A***, A higher percentage of neurons in the D-APV group than in the normal group exhibited surround inhibition. There was no difference in the number of neurons with strong inhibition on one side of the eRF (ASYM), but the number of neurons with inhibition on both sides of the eRF (BOTH) was significantly higher in the D-APV group, and the number with weak inhibition was smaller. ***B***, Paired stimuli were used to determine surround inhibition, with one light spot in the center of the RF and the other at increasing distances from the center on successive trials. The response to the paired stimuli was normalized to the response to a single spot moving through the center of the RF. A decrease in response to the paired stimuli with respect to the single stimulus is interpreted as inhibition. Comparing the surround inhibition between the normal population and the D-APV population showed that inhibition generated by the dual stimuli was stronger in the D-APV group at every location tested except one. The solid bar is the average size of the eRF in the normal group. The open bar is the average eRF size in the D-APV group. $*p < 0.05$.

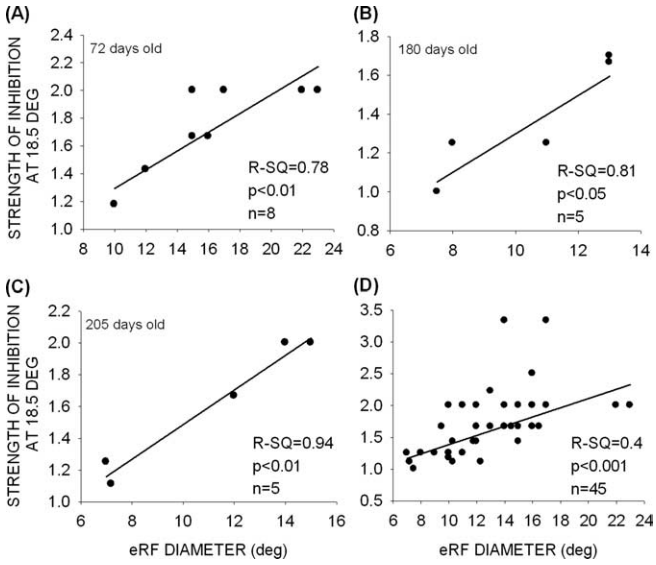

Figure 3. The strength of inhibition at the location farthest from the center of the eRF was correlated with the diameter of the eRF. ***A–C***, Data from three individual hamsters in which the surround and eRF were measured in at least five single neurons. In each case, inhibition at 18.5° was stronger as the eRF diameter was broader. ***D***, Population data from the D-APV group. Although there was considerable variability, the general trend of increasing inhibition with larger eRF diameter was significant. The size of the inhibitory surround was not used in this test because in many D-APV group neurons, the response did not recover to control levels even at 18.5°. Spots beyond 18.5° were not tested because of the monitor size limitations.

normal group. This prediction was tested in the following set of experiments.

The contribution of surround inhibition to velocity tuning in the D-APV group was studied in 29 LP neurons. Similar to LP neurons in the normal group, LP neurons in the D-APV group also depended on inhibition from the NS for velocity tuning. In each of the neurons shown in Figure 4, masking the NS increased the response to rapidly moving stimuli, reducing velocity tuning. As seen with normal LP neurons, many of the D-APV LP neurons still responded better to slowly moving than to rapidly moving stimuli, suggesting that NS inhibition is not sufficient by itself to explain velocity selectivity. Calculation of the percentage of neurons in which velocity tuning depended on surround inhibition revealed that more LP neurons in the D-APV group (17 of 29; 59%) than in the normal group (14 of 38; 37%) (Razak and Pallas, 2005) exhibited an increased response to the nonoptimal, rapidly moving stimuli when the NS was masked (χ^2 test, $p < 0.05$). This was consistent with the finding that more neurons exhibited surround inhibition in the D-APV group (compare Fig. 3). The remaining D-APV neurons showed no effect of surround masking on velocity tuning (Fig. 5).

The amount of release from inhibition caused by masking the NS was compared across the population of LP neurons in both normal and D-APV groups (Fig. 6). Although there was no difference in release at velocities between 30 and 45°/s, the release from inhibition tended to be larger in the D-APV group for velocities between 10 and 25°/s. The difference reached statistical significance for stimuli moving at 10 and 25°/s. Thus, there is an increase in the percentage of LP neurons that depend on surround inhibition for velocity tuning and an increased release from inhibition caused by NS masking at some velocities. This suggests that the NS makes a larger contribution to LP velocity tuning in D-APV neurons than in normal neurons.

In normal SC, surround inhibition is critical for velocity tuning in almost all HP neurons (Razak and Pallas, 2005). The contribution of surround inhibition to velocity tuning was studied in 10 HP neurons in the D-APV group. Masking the TS increased responses to slowly moving stimuli in all HP neurons in the D-APV group (Fig. 7*A–D*). In fact, masking the TS renders each of these HP neurons nonselective for velocity. Masking the NS had no effect. Across the populations of normal and D-APV HP neurons, D-APV neurons showed a larger increase than did normal neurons in response to TS masking for stimuli moving at 5°/s (Fig. 8). There was no significant difference at other velocities.

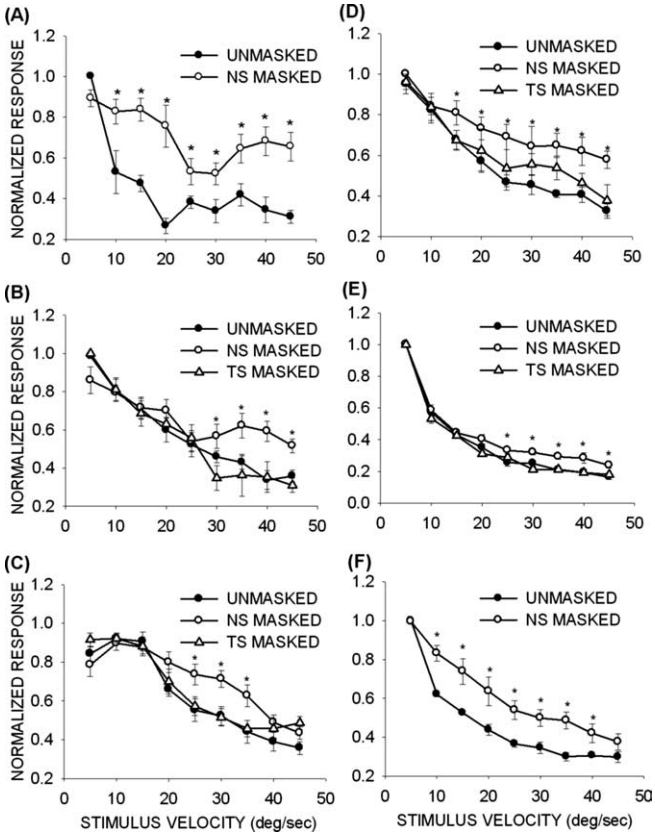

Figure 4. Contribution of surround inhibition to LP velocity tuning in the D-APV group. ***A–F***, Representative LP neurons from D-APV-treated animals in which masking the NS caused a significant increase in response to nonoptimal velocities. ***B–D***, Masking the TS did not cause a systematic change in velocity tuning in LP neurons tested. Stimulus movement for determining velocity tuning was in the temporal–nasal direction. $*p < 0.05$.

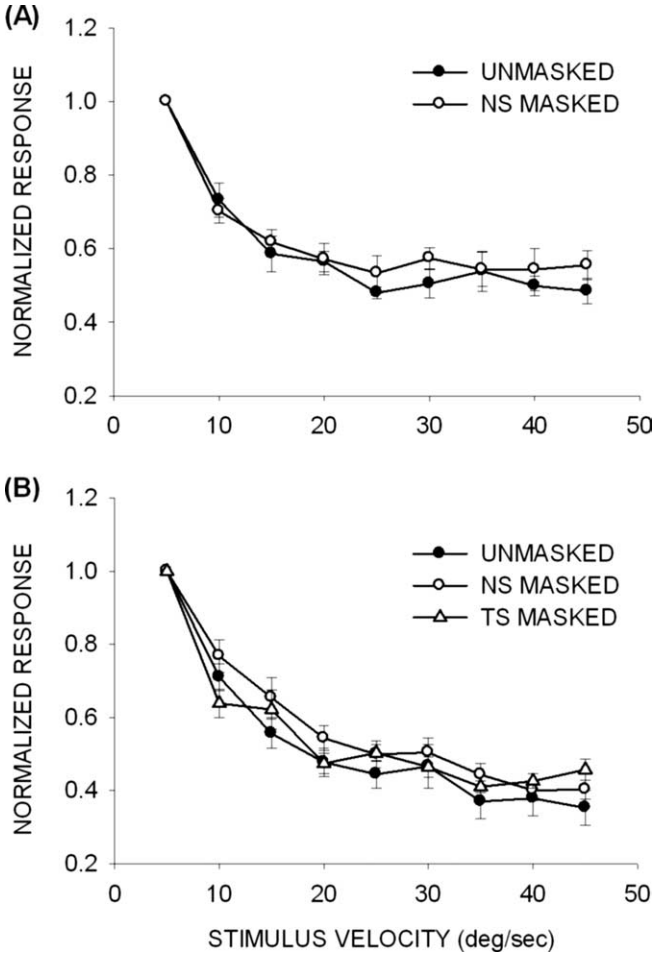

Figure 5. D-APV group LP neurons in which surround masking had no effect on velocity tuning. In these neurons, there was no significant difference ($p > 0.05$) at any velocity between the unmasked and masked response.

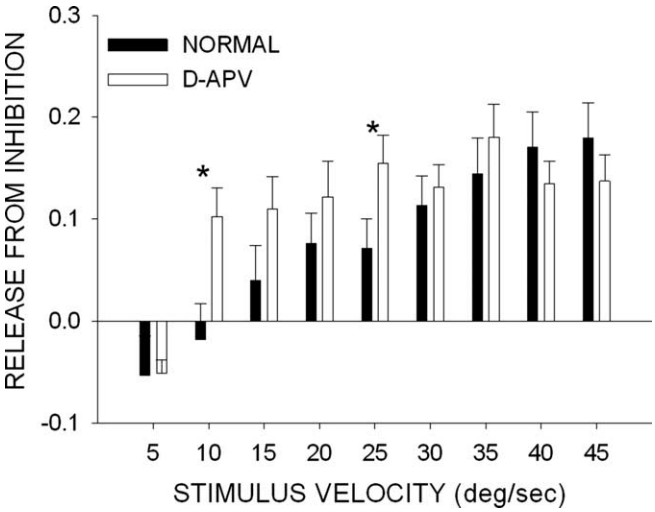

Figure 6. The increase in response to nonoptimal velocities in LP neurons after masking the inhibitory surround was larger in the D-APV group compared with the normal group. The population summary of LP neurons shows that a significant increase in the response after masking occurred for velocities of 10 and 25°/s. The increase in response at other velocities was similar in both groups, peaking at the fastest velocities tested. $*p < 0.05$.

This suggests that the TS inhibits HP neuron responses to slowly moving stimuli to a greater extent in the D-APV group. Together, these results suggest that the increased strength and extent of surround inhibition in the D-APV group contributes to the maintenance of LP/HP velocity tuning after chronic NMDAR blockade and increased eRF diameter.

Discussion

During development or regeneration of the retinal projection to the optic tectum/SC, NMDAR-mediated activity reduces the diameter of the eRF through refinement of retinal axon arborizations, resulting in a reduction in the number of retinal ganglion cells converging on each tectal neuron (Meyer, 1983; Cline and Constantine-Paton, 1989; Schmidt, 1990; Olson and Meyer, 1991; Simon et al., 1992; Hickmott and Constantine-Paton, 1997; Binns and Salt, 1997; Schmidt et al., 2000; Huang and Pallas, 2001) (for review, see Debski and Cline, 2002). The normal, refined size of the eRFs is obtained even if the map is compressed by neonatal partial ablation of caudal SC (Pallas and Finlay, 1989), also through NMDAR-dependent mechanisms; chronic postnatal NMDAR blockade prevents compensation for the map compression and results in enlarged eRF sizes (Huang and Pallas, 2001). Because stimulus velocity selectivity of SC neurons arises from the spatiotemporal relationship between convergent retinal inputs and surround inhibition (Razak and Pallas, 2005), our previous finding that NMDAR blockade-induced eRF enlargement had no effect on tuning to visual stimulus velocity (Razak et al., 2003) was difficult to interpret. In this study, we tested the

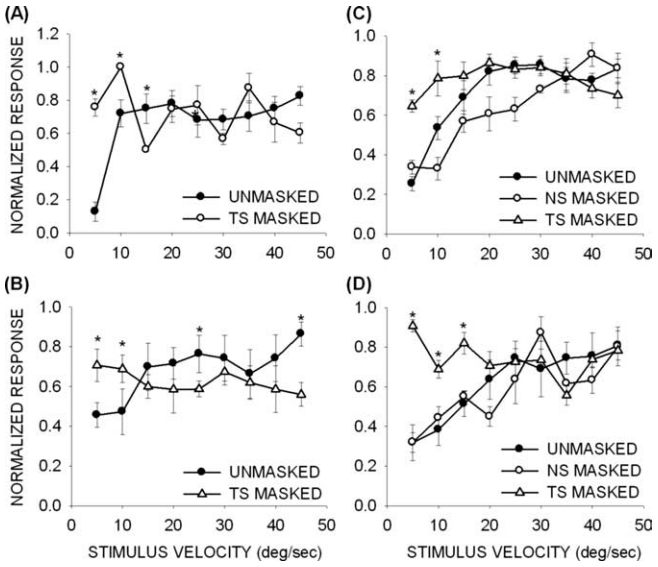

Figure 7. Contribution of surround inhibition to HP velocity tuning in the D-APV group. ***A–D***, Representative HP neurons in which masking the TS caused an increase in response to slow-moving stimuli, resulting in decreased velocity tuning. Masking the NS had no effect on tuning in HP neurons. Stimulus movement for determining velocity tuning was in the temporal–nasal direction. $*p < 0.05$.

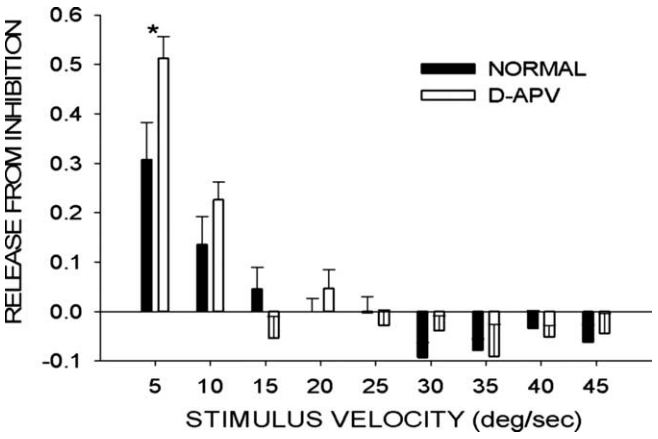

Figure 8. The increase in response to nonoptimal velocities caused by masking in HP neurons was larger in the D-APV group compared with the normal group. Summary of the population of HP neurons shows that the increase in response to stimuli moving at 5°/s was significantly higher in the D-APV group. There was no significant difference at other velocities. $*p < 0.05$.

hypothesis that the preservation of stimulus velocity tuning in NMDAR-blocked, developing retinocollicular maps results from a compensatory, homeostatic rebalancing of the surround inhibition with the expanded excitatory inputs to the eRF. In support of this hypothesis, we have shown here that chronic postnatal NMDAR blockade and the consequent increase in the spatial extent of the RF resulted in a significant and correlated increase in the strength of the inhibitory surround. In addition, a higher percentage of neurons in the D-APV-treated animals exhibited inhibitory surrounds than in normal animals. Surround masking, which reduces velocity tuning in normal animals, reduced it further in neurons from the D-APV group, suggesting that surround inhibition makes a larger contribution to their velocity tuning.

These findings together argue that increased inhibition is responsible for preservation of velocity tuning under conditions of increased excitatory convergence from the retina. Moreover, they show that response properties can be preserved through activity-driven changes in their underlying mechanisms that are not visible without detailed investigation of both excitatory and inhibitory components of neural responses. Thus, knowledge of the circuits that shape response selectivity and how they change during development is necessary to understand how activity influences the development and plasticity of neural response properties.

There are several possible mechanisms that could account for this compensatory rebalancing of excitatory and inhibitory inputs in D-APV-treated SC. Evidence in a number of systems shows that activation of NMDARs can influence the effectiveness of GABAergic inputs. For example, maturation of GABA currents in rat SC may be regulated by NMDAR activity (Shi et al., 1997; Aamodt et al., 2000; Henneberger et al., 2005), and NMDA receptor activation can lead to long-term depression of GABAergic synapses in *Xenopus* tectum (Lien et al., 2006). Thus, changes in the strength of GABAergic synapses can result from the developmental or experimental modification of NMDAR activity.

The apparent changes in strength of the inhibitory surround in our experimental group may result from a homeostatic control mechanism reacting to D-APV-induced perturbations of the balance between excitation and inhibition. Visual deprivation causes a decrease in excitatory synaptic drive from the retina, necessitating a decrease in the inhibitory drive to maintain stable levels of activity in the developing visual cortex (Turrigiano, 1999; Maffei et al., 2004, 2006). A similar homeostatic shift to maintain the balance between excitation and inhibition occurs in the developing auditory cortex after hearing loss (Kotak et al., 2005), the developing external nucleus of the inferior colliculus in barn owls after a prism-induced shift in visual and auditory map registration (Zheng and Knudsen, 1999), and the developing neuromuscular system of activity-deprived chick embryos (Gonzalez-Islas and Wenner, 2006).

However, there are compelling arguments against the idea that the results of this study can be explained by homeostatic plasticity that maintains stable firing levels. In the hamster SC, light-evoked activity is primarily caused by AMPA receptor currents, and chronic NMDAR blockade does not significantly alter levels of glutamate- or light-evoked activity (Huang and Pallas, 2001). Therefore, animals reared with chronic NMDAR blockade may not experience reductions in excitatory SC activity during development. Even if some reduction in activity level could be demonstrated, homeostasis would require a decrease, not an increase, in inhibition (Turrigiano, 1999; Turrigiano and Nelson, 2004). We observed an increase in the strength of surround inhibition. This raises the interesting possibility that the balance between excitation and inhibition can be driven by factors other than maintenance of response levels, such as the spatial extent of excitation.

An alternative explanation for our results is that the increase in the strength and spatial extent of the inhibitory surround could be an indirect effect of chronic NMDAR blockade. In rodent SC, surround inhibition results from the actions of inhibitory interneurons working through $GABA_A$ receptors on the retinorecipient superficial SC neurons (Mize, 1992; Binns and Salt, 1997). These GABAergic interneurons are themselves likely to have a larger eRF as a result of the D-APV-induced increase in retinal convergence. A visual stimulus would thus excite more inhibitory neurons in the D-APV group than normal and could result in the observed increase in the strength and extent of the inhibitory surround (Fig. 9). We propose that this change, in turn, could

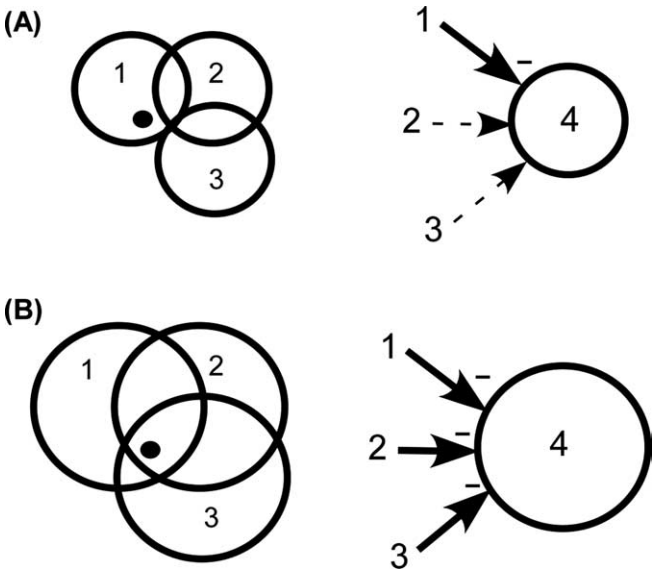

Figure 9. Schematic representation of a possible indirect mechanism through which NMDAR blockade-induced increase in excitatory convergence could result in stronger inhibitory surrounds. Neurons 1–3 are inhibitory interneurons that synapse on neuron 4. The circles represent the excitatory RF of each neuron. The arrows depict the strength of inhibition, with the solid arrow indicating strong inhibition, and the dotted arrow indicating weak inhibition. ***A***, In the normal group, a stimulus (black circle) in the visual field elicits excitatory responses from neuron 1, which in turn provides inhibition to neuron 4. The stimulus does not overlap with the RF of neurons 2 and 3. ***B***, In the D-APV group, NMDAR blockade causes an increase in the eRF size of all 4 neurons. A stimulus in the same location as in ***A*** will excite all three inhibitory neurons. The inhibition on neuron 4 would thus come from a wider area and would likely be stronger.

preserve velocity tuning in compensation for the overafference. This explanation has the added appeal that it provides the system with an automatic, passive compensation for alterations in excitatory input. This compensation mechanism could function during development or evolution or as a mechanism for recovery from abnormal experience or trauma in the SC and perhaps in sensory circuits in general.

Spatiotemporal interactions between excitatory and inhibitory inputs shape response selectivity for several stimulus tuning properties in the visual system (Sillito, 1975) [for review, see Ferster and Miller (2000) and Shapley et al. (2003)]. Previous studies have suggested that the plasticity of inhibitory inputs is critical for both maturation (Humphrey and Saul, 1998; Razak and Pallas, 2006) and maintenance (Carrasco et al., 2005) of visual response properties. Our results present evidence for a mechanism through which inhibitory plasticity is involved in maintenance of response selectivity after manipulation of NMDAR-dependent activity levels during development. Data based on iontophoresis of $GABA_A$ receptor antagonists in the SC shows that velocity tuning is created using local GABA neurotransmission (V. Khoryevin, K. A. Razak, and S. L. Pallas, unpublished results). This suggests that at least some of the modifications in inhibitory circuitry occur locally in the SC as opposed to the retina. The D-APV-treated hamster model is therefore an ideal preparation in which to address the mechanisms underlying plasticity of inhibitory inputs within the context of behaviorally relevant stimulus representation and plasticity.

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