

# Blockade of NMDA Receptors in the Anterior Forebrain Impairs Sensory Acquisition in the Zebra Finch (*Poephila guttata*)

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Juvenile zebra finches (*Poephila guttata*) learn song in two stages: during sensory acquisition, they memorize the song of an adult tutor, and during sensorimotor learning, they alter their vocalizations to match the stored song model. Like many other forms of neural plasticity and memory formation, vocal learning in zebra finches is impaired by pharmacological blockade of NMDA receptors, but the relevant NMDA receptors have not yet been localized. During song development, one neural region that has been implicated specifically in song learning, the IMAN, exhibits an increased density of NMDA receptors as well as decreased binding affinity for the NMDA antagonist MK-801. To test the hypothesis that sensory acquisition requires activation of NMDA receptors in or near the IMAN we infused the NMDA receptor antagonist amino-5-phosphonopentanoic acid (AP5; 2.5  $\mu\text{g}$  in 0.1  $\mu\text{l}$ ) directly into the anterior forebrain. Birds receiving AP5 infusions prior to each of 10 tutoring sessions copied significantly less of their tutor's song than did sham-operated birds, saline-infused birds, birds that received AP5 infusions on nontutoring days, or birds that received AP5 infusions into the cerebellum. Furthermore, infusions of AP5 in the anterior forebrain did not impair young birds' ability to discriminate zebra finch from canary song. These findings are consistent with the hypothesis that NMDA receptor activation in the anterior forebrain is necessary for the memorization of song material during avian vocal learning. This is also the first report that song-related regions of the anterior forebrain contribute to sensory acquisition specifically. © 1996 Academic Press, Inc.

The *N*-methyl-D-aspartate (NMDA) receptor is implicated in various aspects of neural plasticity including memory formation. This receptor is unique in that coincidence between presynaptic release of

neurotransmitter and postsynaptic depolarization permits  $\text{Ca}^{2+}$  influx triggering a biochemical cascade that can lead to long-term changes in synaptic strength (Malenka & Nicoll, 1993; Mayer & Westbrook, 1987; Nowak, Bregestovshi, Ascher, & Pronchiantz, 1984). In this way, NMDA receptors function as a component of "Hebbian" synapses where changes in synaptic efficacy reflect the prior history of coupling between presynaptic activity and postsynaptic activation. Presumably, these Hebbian-type synapses provide a neural substrate for memory formation. Pharmacological blockade of NMDA receptors compromises long-term potentiation (Collingridge, Kehl, & McLennan, 1983; Harris, Ganong, & Cotman, 1984; Nicoll & Malenka, 1995), spatial learning (Morris, Davis, & Butcher, 1990), learned taste aversion (Burchuladze & Rose, 1992; Steele & Stewart, 1993), acquisition and extinction of conditioned fear (Falls, Miserendino, & Davis, 1992; Kim & McGaugh, 1992; Liang, Hon, & Davis, 1994), early olfactory learning (Lincoln, Coopersmith, Harris, Cotman, & Leon, 1988), and the experience-dependent modification of neuronal properties in kitten visual cortex (Bear, Kleinschmidt, Gu, & Singer, 1990; Gu, Bear, & Singer, 1989; Kleinschmidt, Bear, & Singer, 1987). In addition, it has been shown recently that song learning in one species of songbird, the Australian zebra finch (*Poephila guttata*) requires activation of NMDA receptors (Aamodt, Nordeen, & Nordeen, 1996).

Zebra finches, like many passerine songbirds, learn songs only during a discrete developmental period. Songs are learned in two distinct phases, each of which requires auditory experience. During the first phase, sensory acquisition, birds memorize song material produced by other adult males (Koniishi, 1965; Marler, 1970; Marler & Peters, 1977; Thorpe, 1958). In zebra finches, learned songs are produced exclusively by males, and they normally

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imitate only those songs heard between about 20 and 60 days after hatching (Böhner, 1990; Eales, 1985, 1987; Immelmann, 1969). Birds that do not have the opportunity to learn from an adult song tutor during this period produce an "isolate type" song that is characterized by unusual syllable types and phonology (Immelmann, 1969; Marler, 1970; Williams, Kilander, & Sotanski, 1993). During the second phase of song learning, sensorimotor learning, birds begin to produce song-like vocalizations and use auditory feedback to match their own song to the memorized song model. Birds deafened before this stage of learning produce songs of even poorer quality than those of isolate birds (Konishi, 1965; Marler, Mundinger, Waser, & Lutjen, 1972; Nottebohm, 1968; Price, 1979). Zebra finches begin rehearsing song about 35 days after hatching, and by 90–120 days crystallize a stereotyped song pattern that normally remains stable throughout adult life.

Blocking NMDA receptors during sensory acquisition interferes with the normal development of song behavior in zebra finches. Aamodt et al. (1995) found that systemic injections of the NMDA receptor non-competitive antagonist MK-801 immediately before tutoring sessions impaired song development, yet identical injections delivered between tutoring sessions produced no impairment. These results suggested that vocal learning in zebra finches requires the activation of NMDA receptors during song memorization. However, while MK-801 injections did not alter the auditory brainstem response to simple tones, it was not clear from this earlier study whether the disruptions of song learning produced by MK-801 reflected NMDA receptor involvement in the encoding and storage of song-related memories as opposed to the auditory processing necessary for recognizing conspecific song. In addition, because MK-801 injections were administered systemically in this experiment, the location of the NMDA receptors contributing to song learning could not be evaluated.

Relevant to this latter issue, several regions of the avian brain have been directly implicated in song behavior (see Fig. 1). Song production is controlled by a pathway that includes the high vocal center (HVC) in the neostriatum and the robust nucleus of the archistriatum (RA); it is not known whether these regions also are involved directly in song learning (Nottebohm, Stokes, & Leonard, 1976; Simpson & Vicario, 1990). In contrast, an anterior forebrain circuit that includes the Area X, the medial portion of the dorsolateral nucleus of the anterior thalamus (DLM), and the lateral magnocellular nucleus of the anterior neostriatum (IMAN) is involved

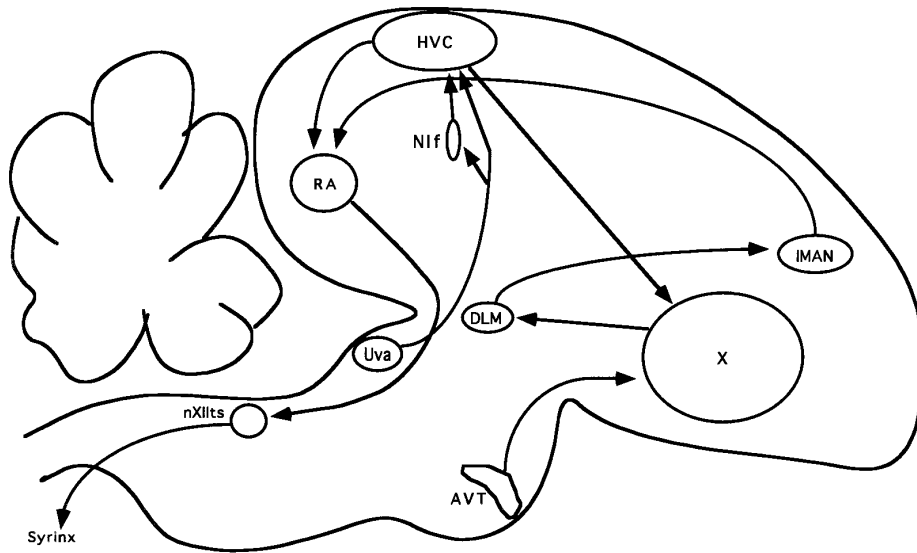
specifically in song learning, but not the production of adult song. Destroying any of these areas in juveniles precludes normal song development, whereas lesioning in adulthood has no effect on song production (Bottjer, Miesner, & Arnold, 1984; Halsema & Bottjer, 1991; Nordeen & Nordeen, 1993; Scharff & Nottebohm, 1991; Sohrabji, Nordeen, & Nordeen, 1990).

Two observations suggest that NMDA receptors involved in sensory acquisition may reside within the anterior forebrain song circuit. First, as noted above, this forebrain loop has been implicated specifically in normal song development. Second, one region within this circuit, the IMAN, exhibits changes in NMDA receptor binding that correlate with song learning. Between 30 days after hatching and adulthood, when juveniles are memorizing song material and beginning to rehearse that material in their own vocalizations, there is a gradual decline in binding density as well as an increase in binding affinity for MK-801 (Aamodt et al., 1996). Coupled with the behavioral impairments that follow systemic injections of MK-801, this developmental change in ligand binding suggests that NMDA receptors in the IMAN may play an important role in mediating the effects of auditory experience on vocal development. To test the hypothesis that sensory acquisition requires activation of NMDA receptors in anterior forebrain components of the song system, we infused the NMDA receptor competitive antagonist amino-5-phosphonopentanoic acid (AP5) directly into the anterior forebrain immediately prior to exposure to a song tutor. We report that blockade of these NMDA receptors during acquisition impairs vocal learning without compromising the ability of birds to discriminate zebra finch from canary song.

## EXPERIMENTAL DESIGN AND METHODS

### *Experiment 1—Effects of AP5 Infusions on Song Development*

Zebra finch chicks were bred and raised in our laboratory and maintained on a 14:10 light:dark cycle. Within 8 days after hatching, chicks were removed from the breeding aviary and placed in a cage with both parents. The following day, the father was removed and the chicks were raised by the mother in a room isolated from conspecific song. At 30 days of age, juvenile females were removed from the experiment and males were assigned randomly to one of five groups; sham operates, experimental birds (AP5), AP5 controls (CNTL<sub>AP5</sub>), cerebellum-infused controls (Cb), and saline controls. Sham-operated



**FIG. 1.** Sagittal schematic view of the avian brain showing regions that have been implicated directly in song learning and production. Abbreviations: ventral area of Tsai (AVT); the medial portion of the dorsolateral nucleus of the anterior thalamus (DLM); high vocal center (HVC); the lateral magnocellular nucleus of the anterior neostriatum (IMAN); nucleus interfacialis (Nif); the robust nucleus of the archistriatum (RA); Area X; the tracheosyringeal portion of the 12th cranial nerve nucleus (nXIIts).

birds were anesthetized with Equithesin and a mid-line incision was made through the skin overlying the skull. The incision was immediately closed with a flexible plastic adhesive (collodion) and the bird allowed to recover. All other males were implanted bilaterally with 28-gauge guide cannulae directed at either the IMAN (AP5, CNTL<sub>AP5</sub>, and saline groups) or the cerebellum. Using stereotaxic coordinates previously established in our lab, these guide cannulae were placed just dorsal to the target area to insure that the 31-gauge infusion cannulae extruded into the target region. Following surgery all birds were housed in individual cages, visually isolated from other males except during tutoring sessions. Such visual isolation effectively prevents birds from imitating song elements produced by males that can be heard but not seen (Eales, 1989; personal observations).

Between 32 and 52 days of age birds spent 90 min every other day housed together in a large cage (50 × 50 × 50 cm) with a testosterone-implanted adult male tutor and his mate. This restricted tutoring regime allowed us to block NMDA receptors during opportunities for song memorization, while minimizing pharmacological interference with vocal practice. We have used a similar tutoring regime previously and found it to be sufficient for normal vocal learning (Aamodt et al., 1996). To block NMDA receptors, birds in the AP5 group received bilateral 0.1  $\mu$ l infusions of 127 mM AP5 5–20 min prior to tutor presentation. We infused the competitive antagonist AP5

because studies have shown that intracranial infusions of AP5 impair many types of memory formation including spatial memory, odor aversion, and imprinting (McCabe, Davey, & Horn, 1992; Morris, Anderson, Lynch, & Baudry, 1986; Willner, Gallagher, Graham, & Crooks, 1992). In rats, 0.5  $\mu$ l intra-amygdala infusions of approximately 100 mM D,L-AP5 blocks retention of an inhibitory avoidance task (Kim & McGaugh, 1992) as well as the acquisition (Campeau, Miserendino, & Davis, 1992) and extinction (Falls et al., 1992) of a fear-potentiated startle response. The saline control group received saline infusions into the IMAN just prior to tutoring. The CNTL<sub>AP5</sub> group received identical bilateral infusions of AP5 on nontutor days to control for effects of AP5 that might be related to processes distinct from acquisition (i.e., interference with sensorimotor learning, tissue damage). To test for the regional specificity of the pharmacological manipulation, a placement control group received the same dose of AP5 in the cerebellum prior to tutoring. All tutoring sessions and infusions took place within 2 h into the light phase of the cycle. Juveniles were tutored in five groups that ranged in size from 5 to 15 birds. Within a tutoring group the age range did not exceed 10 days and each group consisted of birds from the experimental and at least two of the four control groups.

At 90 days of age, each bird was placed in view of a stimulus female and directed song was recorded (P2 Fender microphone, Mirantz PMD cassette re-

order). The birds then were sacrificed by lethal injection of Equithesin and intracardially perfused with saline and formalin. Brains were cryoprotected in a 30% sucrose solution and then frozen 40- $\mu$ m sections were mounted onto slides and stained with thionin to verify the placement of the infusion cannulae.

Adult zebra finch song consists of 2–10 syllables organized into a phrase that is repeated several times during each song bout. Several song bouts from each bird and their tutor were analyzed to obtain two measures of learning: (1) the percentage of the tutor's syllables copied by the subject and (2) the percentage of the subject's syllables that were improvised (i.e., not obviously copied from the tutor). Sonograms were produced on a Kay Digital Sonograph (DSP 5000) according to methods used routinely in our laboratory (Aamodt, Nordeen, & Nordeen, 1994; Nordeen & Nordeen, 1992; Nordeen & Nordeen, 1993; Sohrabji et al., 1990). Individual syllables were defined as acoustic units lasting at least 20 ms and surrounded by intervals of baseline energy lasting at least 10 ms, except in cases of abrupt frequency transitions (>1 kHz), where the intersyllable interval could be as short as 5 ms. Using the sonograms, each song syllable in an experimental bird's song was matched to the syllable in the tutor's song that it most resembled. The similarity of the pair was scored on a 0 to 3 scale (0 = no similarity, 1 = slightly similar, 2 = highly similar, 3 = matched). Two experimenters blind to the subjects' treatment independently analyzed the sonograms and these scores then were averaged (agreement between scorers was 92%). Learned syllables were operationally defined as those that received a score  $\geq 2.0$ . The remaining syllables were operationally defined as being improvised. A third experimenter scored each song for the presence or absence of "isolate type" song elements such as repeated syllables within a phrase, abnormally long syllables, high-frequency whistles, frequency upsweeps, and clicks. Because zebra finch syllables often contain frequency-modulated complex harmonic structures, we have found that visual comparison of song syllables generates more reliable results than computer-based autocorrelation programs (see also Williams et al., 1993). The mean sonogram scores for each experimental group were calculated and Mann–Whitney *U* tests used to test for differences between group means. Because we have found previously that systemic blockade of NMDA receptors during tutoring disrupt normal song development (Aamodt et al., 1996), we hypothesized that IMAN directed infusions of AP5 prior to tutoring would also impair vocal

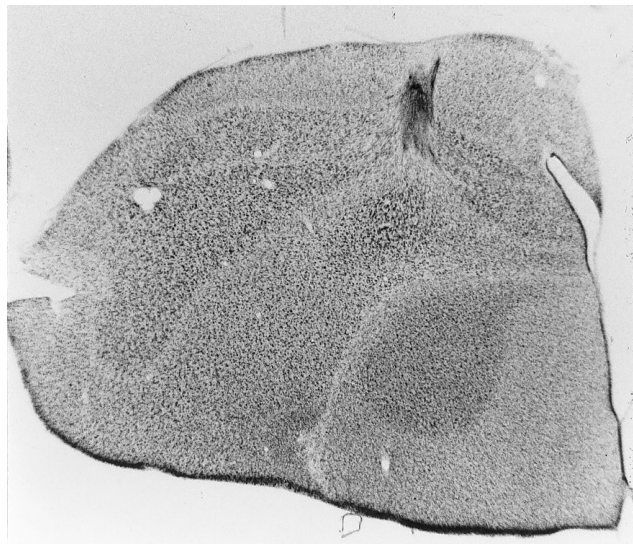
learning. Therefore, for the number of learned syllables a one-tailed test was used to compare the experimental group with the saline and CNTL<sub>AP5</sub> groups; all other comparisons used two-tailed tests.

### *Experiment 2—Effects of AP5 Infusions on Song Discrimination*

A second group of zebra finches was used to determine whether AP5 infusions into the anterior forebrain disrupt song discrimination. Chicks were raised in our breeding aviaries and at 30 days of age juvenile males were implanted with bilateral cannulae directed at the IMAN as described above. Within the next 20 days, each bird's ability to discriminate zebra finch from canary song was tested before and after infusions of AP5. Discrimination testing was conducted in a 180 cm (l)  $\times$  30 cm (h)  $\times$  45 cm (d) rectangular chamber with perches placed in the middle and 20 cm from each end. Each 10-minute trial began with the zebra finch on the center perch and recordings of zebra finch song and canary song played continuously through speakers located at each end of the chamber. A sound pressure meter was used to ensure that the intensity of each song stimulus was similar at the beginning of each trial. The mean intensity of the stimuli measured 20 cm from the speaker was 98.4 dB. The song type presented through each speaker varied randomly across trials. The number of minutes spent within 20 cm of either speaker (referred to as the approach zone) was recorded for each trial. During their first trial, 5 of 10 birds tested spent greater than 7 min within the zebra finch approach zone. The other birds spent less than 1 min within either approach zone. Those birds showing a clear preference were retested 15 and 60 min after intracranial infusions of AP5 (2.5  $\mu$ g in 0.1  $\mu$ l saline) directed toward the IMAN. Following these three trials, birds were sacrificed by lethal injection of Equithesin, intracardially perfused with saline and formalin, and the brains processed as described above to verify the placement of the infusion cannulae.

## RESULTS

In all the animals used for analysis the ventral extent of the cannula damage was located either just dorsal to the IMAN or within the cerebellum. Several animals were excluded from the analysis because the IMAN had been damaged. The final sample size for each group was: 7 AP5, 8 CNTL<sub>AP5</sub>, 7 cerebellum, 7 saline, and 7 sham-operate birds in Experiment 1 and 5 birds in Experiment 2. A representative thio-

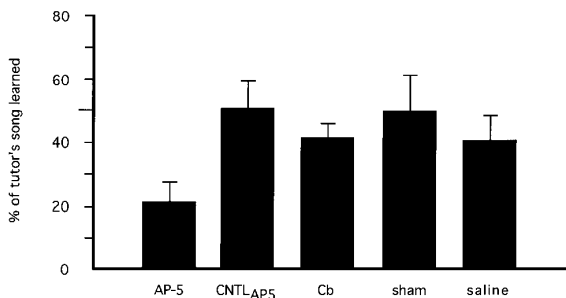


**FIG. 2.** A representative thionin-stained coronal section from a bird with a cannula directed at the IMAN.

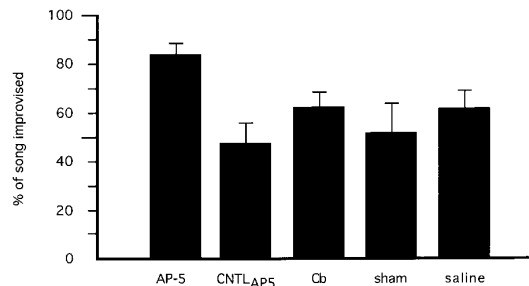
nin-stained section from a bird with a cannula in the anterior forebrain is shown in Fig. 2.

The highly restricted tutoring regime used in the present experiment (10 90-min sessions) provided sufficient opportunity for the storage of song-related information. Birds in the sham control group copied 49.6% of the tutor's song, an amount not significantly different than the 50.9% we observed in eight male birds isolated until 30 days of age and then raised in constant contact with adult tutors. Infusions of saline into the anterior forebrain just prior to tutoring did not affect significantly the amount of song material copied from the tutors. The saline control group copied only slightly less of the tutor's song than did the sham control birds (see Fig. 3).

As shown in Fig. 3, the effects of AP5 infusion into the anterior forebrain depended upon when they were made relative to the tutoring sessions. Birds



**FIG. 3.** Percentage of the tutor's song learned by 90 days of age. When delivered just prior to song tutoring, AP5 infusions directed toward the IMAN (AP5 group) significantly reduced the amount of song material learned.



**FIG. 4.** Percentage of the bird's own song that was improvised (not copied from the tutor). Birds infused with AP5 into the IMAN just prior to tutoring improvised significantly more of their song than did birds in the other groups.

that received AP5 just prior to being placed with the tutor copied significantly fewer syllables than did birds infused with saline ( $z = 1.9$ ;  $p \leq .03$ , one-tailed) or those infused with AP5 on nontutoring days ( $z = 2.4$ ;  $p \leq .02$ , two-tailed). In contrast, infusions of AP5 on the nontutoring days did not noticeably impair vocal learning. That is, the percentage of the tutor's song copied by the CNTL<sub>AP5</sub> group was not significantly different from the saline group.

The effects of AP5 infusions on song learning were regionally specific. In contrast to infusions into the anterior forebrain, AP5 infusions into the cerebellum just prior to tutoring did not impair vocal learning. As shown in Fig. 3, the percentage of the tutor's song copied by the cerebellum group was not significantly different from the sham group but was significantly greater than the AP5 group ( $z = 2.1$ ;  $p \leq .04$ , two-tailed).

Although birds infused with AP5 shortly before tutoring copied relatively little from the tutor, there were no significant group differences in the number of syllables produced. As shown in Fig. 4, this was because the AP5 group produced significantly more improvised syllables than did the saline control group ( $z = 2.3$ ;  $p \leq .02$ , two-tailed), the CNTL<sub>AP5</sub> group ( $z = 3.1$ ;  $p \leq .002$ , two-tailed), or the cerebellum control group ( $z = 2.4$ ;  $p \leq .02$ , two-tailed). For all groups, most of the improvised syllables were normal in their morphology, and there were no significant group differences in the incidence of isolate-type syllables (e.g., syllables with abnormal length, high-frequency whistles, frequency upsweeps, or clicks). In both the AP5 and saline control group three of seven birds produced improvised syllables that were abnormally long in duration, and in the CNTL<sub>AP5</sub> group, one of eight birds produced isolate-type elements.

Infusions of AP5 into the anterior forebrain did not impair the bird's ability to distinguish zebra

**TABLE 1**  
**Effects of Intracranial AP5 Infusions on Ability to Discriminate**  
**between Zebra Finch Song and Canary Song**

	Mean $\pm$ SEM	
	Percentage of time spent in zebra finch approach zone	Percentage of time spent in canary approach zone
Baseline	74.0 $\pm$ 10.5	0.9 $\pm$ 0.6
15 min postinfusion	61.9 $\pm$ 7.4	0.3 $\pm$ 0.3
60 min postinfusion	61.2 $\pm$ 9.1	5.7 $\pm$ 3.3

finch from canary song (Table 1). Under baseline conditions, birds that made a choice in the discrimination testing paradigm spent  $74.0 \pm 10.5\%$  of the test time in the zebra finch approach zone and virtually no time in the canary approach zone ( $0.9 \pm 0.6\%$  of total time). This preference was not altered significantly by infusions of AP5 through cannulae directed toward the IMAN. Fifteen minutes after infusion, birds spent  $61.9 \pm 7.4\%$  of the test time in the zebra finch approach zone and 60 min after infusion they spent  $61.2 \pm 9.1\%$  of their time in this zone. In addition, birds still spent very little time in the canary approach zone after infusions of AP5; only  $2.4 \pm 1.5\%$  of total postinfusion test time was spent in this zone after AP5 infusions. The infusions of AP5 also did not alter the birds observable repertoire of behaviors. At all test times birds oriented toward, and often pecked at, the speaker playing zebra finch song.

## DISCUSSION

The results of this experiment support the hypothesis that NMDA receptors in the anterior forebrain song circuitry participate in normal song development. When delivered just prior to tutoring, AP5 infusions directed toward the IMAN significantly reduced the amount of song material copied from the tutor, whereas similar infusions delivered into the cerebellum did not. Although our cannula placement in the anterior forebrain was chosen so as to target NMDA receptors within the IMAN, we cannot assume that our infusions were limited to this region. It is likely, however, that the functional diffusion of AP5 was less than 2.0 mm from the cannula tip. This estimate is based on a study by Liang et al. (1994) in which a  $0.5\text{-}\mu\text{l}$  infusion of 25 nmol AP5 into the amygdala of rats impaired memory on a inhibitory avoidance task, whereas control infusions 2.0 mm dorsal to the amygdala (into the striatum) did not. In order to restrict the range of our pharma-

cological manipulation, we injected one-half of this molar dose in a volume of only  $0.1 \mu\text{l}$ . Thus, our effective range of diffusion was probably less than that in the Liang study. Aside from the IMAN, Area X is the only other nucleus implicated in song behavior that was in close proximity to our forebrain cannula. In fact, all other song-related nuclei were closer to our cerebellar infusions (5.7 mm posterior to the IMAN), which produced no deficits in song learning. Area X is approximately 1.7 mm ventral to the site of our anterior forebrain infusions and although it is separated from the IMAN by a large fiber tract (which may impede diffusion) it is possible that our AP5 infusions blocked NMDA receptors within this region along with those in the IMAN. Both of these areas contain moderate to high levels of NMDA receptors (Aamodt, Kozlowski, Nordeen, & Nordeen, 1992), and both participate in avian song development.

While we believe that the behavioral impairments seen in the present study are due to the blockade of NMDA receptors in the anterior forebrain, NMDA receptors within other parts of the song system may also contribute to song learning. In this regard, it is interesting that the disruptions of song structure seen in the present study were not as severe as those that occur following systemic injections of an NMDA receptor antagonist (Aamodt et al., 1996). NMDA receptors are present in both RA and HVC (Aamodt et al., 1992), and in RA they mediate fast transmission from the IMAN (Kubota & Saito, 1991; Mooney, 1992; Mooney & Konishi, 1991). Although we do not know how the RA might participate in song learning, infusions of AP5 into this region disrupt song production in adult zebra finches (Lombardino & Nottebohm, 1993).

Although normal song development involves both sensory acquisition and sensorimotor learning, the behavioral impairments observed in this study most likely reflect a specific disruption of sensory acquisition. Thus, while lesion studies have shown both

Area X and the IMAN to be necessary for normal song development, the results of the present experiment are the first to implicate one or both of these regions specifically in sensory acquisition. We base this conclusion on the observation that AP5 reduced the amount of song material learned only when delivered into the anterior forebrain just prior to tutoring. Identical infusions on nontutoring days did not produce the same detrimental effects. Thus, even if AP5 did transiently disrupt vocal practice, the design of the experiment apparently allowed sufficient vocal practice either between AP5 infusions or after 52 days of age for song behavior to develop normally. Of course, this conclusion does not preclude the possibility that the anterior forebrain song circuit also participates in sensorimotor learning, when young birds use auditory feedback to gradually match their own vocalizations to the song memories stored earlier in life. In fact, the IMAN and Area X are likely also involved in this later stage of vocal development. Even after sensory acquisition is complete, lesions of the IMAN or Area X made during sensorimotor learning result in abnormal song patterns, and the effectiveness of these lesions declines as adult song patterns develop (Bottjer et al., 1984; Scharff & Nottebohm, 1991). It will be important to determine in future studies if NMDA receptors are involved also in this latter phase of song development.

Although our data provide strong evidence that NMDA receptors participate in normal sensory acquisition, we cannot attribute the observed behavioral deficits directly to disruption of synaptic processes that mediate memory formation. That is, similar behavioral impairments might occur if NMDA receptors in the anterior forebrain contribute to general neuronal responsiveness within the song system. If AP5 reduces normal transmission below a critical threshold value, cellular cascades required for memory formation may not be initiated. In several different systems, evoked responses to sensory input are attenuated by NMDA antagonists (see Daw, Stein, & Fox, 1993, for review), and this has complicated the interpretation of many experiments designed to evaluate the role of NMDA receptors in experience-dependent synaptic modifications (Bear et al., 1990; Miller, Chapman, & Stryker, 1989). Unfortunately, we do not know if NMDA receptors within the anterior forebrain song system are involved in fast transmission. However, within the RA, NMDA receptors apparently mediate the bulk of transmission through IMAN-RA synapses (Kubota & Saito, 1991; Mooney & Konishi, 1991). In this same region, HVC inputs utilize primarily non-NMDA

glutamate receptors for synaptic transmission, but an NMDA receptor-mediated component to the EPSP is revealed when postsynaptic membranes are sufficiently depolarized (Perkel, 1994). It is also interesting that infusions of AP5 directed toward RA disrupt adult song production even if the IMAN has previously been lesioned, suggesting that normal information processing through the vocal motor pathway requires the activation of NMDA receptors within the vicinity of RA (Lombardino & Nottebohm, 1993). Before we can resolve how NMDA receptors in the anterior forebrain participate in sensory acquisition, it will be important to determine whether NMDA receptor blockade diminishes either spontaneous or evoked activity of IMAN or Area X neurons.

It is also possible that the impairments of song development produced by NMDA receptor blockade reflect more generalized deficits in behavioral processes required for learning. Specifically, if AP5 infusions disturb the fidelity of acoustic processing, birds may not recognize the tutor's song as being suitable for sensory acquisition. This issue is of particular concern because songbirds are highly selective in what they choose as a model for vocal imitation (Eales, 1987; Marler, 1970; Marler & Peters, 1977; Thorpe, 1958). A second possibility is that AP5 interferes with attention or motivational systems. These systems likely modulate activity within the zebra finch song system, since juveniles learn best from live tutors with which they can interact both vocally and visually (Eales, 1985, 1989). One strategy that can circumvent these potential problems is to deliver NMDA antagonists immediately following training. Although this approach has been used successfully (Burchuladze & Rose, 1992; Liang et al., 1994; Ungerer, Mathis, Melan, & De Barry, 1991), it is primarily suited to learning paradigms wherein the training trials can be very brief so that pharmacological manipulations can be made before the biochemical cascades involved in memory formation are firmly established. Pilot studies in our lab suggest that acquisition in zebra finches does not occur readily with brief tutoring sessions. Birds tutored for 20 min every other day between 32 and 52 days of age exhibited vocal learning so poor that a decrease due to pharmacological manipulation would be impossible to detect. Therefore, an alternative approach to address these issues was employed in Experiment 2 in which we tested the effects of AP5 on a task that required complex sensory processing and engaged motivational systems, but did not require memory storage. The results of this experiment suggest that the learning impairments produced by AP5 infusions were not due to disruptions of auditory

processing, attention, or motivation. That is, the same concentration and dose of AP5 that disrupted sensory acquisition did not impair the bird's ability to discriminate between zebra finch and canary song. Moreover, because AP5 infusions did not alter qualitatively the behavior of birds during these discrimination tests, it is unlikely that NMDA receptor blockade grossly disrupted attention or motivation.

Most current models of memory storage suggest that experience is stored by the selective strengthening of neuronal circuits, and there is abundant evidence that NMDA receptors can mediate such changes in synaptic efficacy (see Bliss & Collingridge, 1993, for review). In the case of so-called "sensitive" periods in neural and behavioral development, increased propensity for experience-dependent plasticity is often associated with a period of collateral retraction and/or synapse elimination. In many of these cases, NMDA receptors participate in the activity-driven reorganization of synaptic connections. For example, NMDA receptors are involved in terminal retraction during the development of ocular dominance patterns in mammalian visual cortex (Bear et al., 1990) and lateral geniculate (Hahm, Langdon, & Sur, 1991), retinotopic sharpening in goldfish tectum (Schmidt, 1990), and elimination of climbing fiber synapses onto Purkinje cells in the cerebellum (Rabacchi, Bailly, Delhay-Bouchaud, & Mariani, 1992). Moreover, in several of these systems, a critical period for synaptic plasticity is associated with unique patterns of NMDA receptor expression and function (Bode-Greuel & Singer, 1989; Carmignoto & Vicini, 1992; Fox, Daw, Sato, & Czepita, 1991; Fox & Daw, 1993; Hofer & Constantine-Paton, 1994). In view of these findings, it is interesting that during sensory acquisition, the size of the terminal field from the DLM onto the IMAN decreases (Johnson & Bottjer, 1992), as does the frequency of dendritic spines on IMAN neurons (Nixdorf-Bergweiler, Wallhäusser-Franke, & DeVoogd, 1995). Moreover, treatments that extend the sensitive period for sensory acquisition also delay the developmental reduction in dendritic spine density within the IMAN (Wallhäusser-Franke, Nixdorf-Bergweiler, & DeVoogd, 1995). Significantly, this apparent synaptic reorganization is accompanied by a developmental decline in the density of NMDA receptors within the IMAN as well as probable changes in the functional properties of these receptors (Aamodt et al., 1992, 1994). Thus, it is tempting to speculate that NMDA receptors within the IMAN may participate in the encoding or storage of song-specific memories through the selective retention of specific synapses. Future studies assessing how NMDA re-

ceptor antagonists affect developmental changes in spine density among IMAN neurons as well as further elucidating the pattern of developmental changes in NMDA receptor function in zebra finches will address this hypothesis more directly.

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