

Developmentally Restricted Synaptic Plasticity in a Songbird Nucleus Required for Song Learning

Charlotte A. Boettiger^{1,2} and Allison J. Doupe¹

Keck Center for Integrative Neuroscience
Neuroscience Graduate Program and
Sloan-Swartz Center for Theoretical Neurobiology
Departments of Physiology and Psychiatry
University of California, San Francisco
San Francisco, California 94143

Summary

We provide evidence here of long-term synaptic plasticity in a songbird forebrain area required for song learning, the lateral magnocellular nucleus of the anterior neostriatum (LMAN). Pairing postsynaptic bursts in LMAN principal neurons with stimulation of recurrent collateral synapses had two effects: spike timing- and NMDA receptor-dependent LTP of the recurrent synapses, and LTD of thalamic afferent synapses that were stimulated out of phase with the postsynaptic bursting. Both types of plasticity were restricted to the sensory critical period for song learning, consistent with a role for each in sensory learning. The properties of the observed plasticity are appropriate to establish recurrent circuitry within LMAN that reflects the spatiotemporal pattern of thalamic afferent activity evoked by tutor song. Such circuit organization could represent a tutor song memory suitable for reinforcing particular vocal sequences during sensorimotor learning.

Introduction

Long-lasting changes in the strength of synaptic connections are widely thought to be one physiological mechanism underlying learning (Hebb, 1949; Martin et al., 2000). Studies of song learning in birds have several advantages for investigating this hypothesis. Song learning in the zebra finch is a well defined behavior that takes place in two stages: a sensory critical period when the memory of a song model (or “tutor” song) is stored, and a sensorimotor phase, during which vocal output is gradually matched to the stored memory of that tutor song (Immelmann, 1969) (Figure 1A). In addition, song learning requires the function of a discrete network of brain areas known as the song system (Nottebohm et al., 1976) (Figure 1B). The lateral portion of the magnocellular nucleus of the anterior neostriatum (LMAN), which projects to the motor control pathway for song (Nottebohm et al., 1982; Okuhata and Saito, 1987; Bottjer et al., 1989; Mooney and Konishi, 1991), may be one site of neural changes underlying learning. Lesions of LMAN during song acquisition perturb song development, whereas similar disruptions in adult birds do not affect normal song production (Bottjer et al., 1984; Soh-

rabji et al., 1990; Scharff and Nottebohm, 1991; Nordeen and Nordeen, 1992; Morrison and Nottebohm, 1993).

LMAN has been implicated in processing sensory information related to both the tutor song and the bird's own song (BOS) and in providing an evaluation to the vocal motor system of how well the BOS matches the tutor song. This idea is based on the fact that selective tuning of LMAN neurons for the sound of the tutor and BOS emerges during the course of song learning (Doupe, 1997; Solis and Doupe, 1999, 2000) and that lesions of LMAN prevent the degradation of song normally caused by perturbations of song production or feedback (Williams and Mehta, 1999; Brainard and Doupe, 2000). Moreover, blockade of NMDA receptors (NMDARs) in LMAN during tutoring prevents birds from producing a good copy of the tutor song (Basham et al., 1996), consistent with LMAN contributing to tutor song memorization. This result, coupled with the significant downregulation of NMDAR expression in LMAN by the end of the sensory critical period (Aamodt et al., 1992), suggests the hypothesis that NMDAR-dependent long-term plasticity is present in LMAN during sensory learning.

We investigated this hypothesis using an *in vitro* zebra finch brain slice preparation containing LMAN. Our results show that there is a spike timing and NMDAR-dependent form of long-lasting synaptic potentiation, or LTP, in LMAN. This LTP was observed at the recurrent excitatory (LMAN_R) synapses, compatible with our previous observation that the recurrent synapses of LMAN principal cells are NMDAR rich (Boettiger and Doupe, 1998), and appears similar in several respects to mammalian neocortical LTP (Crair and Malenka, 1995; Kirkwood et al., 1995; Hensch et al., 1998; Feldman, 2000). Moreover, the LTP of the recurrent synapses was limited to the critical period for memorization of tutor song. We found, in addition, that the protocol to induce LTP of the LMAN_R synapses also induced a long-lasting depression (LTD) at thalamic input synapses to LMAN, and that this LTD was restricted to younger animals as well. These forms of developmentally restricted long-term plasticity are well suited to contribute to the experience-dependent shaping of auditory responses in LMAN and to the memorization of tutor song.

Results

Using an anterior forebrain slice preparation from zebra finches early in sensory learning (20 days) or at the end (60 days) of the critical period for sensory learning (Figure 1A), we made intracellular voltage recordings from LMAN principal neurons. We recorded synaptic responses to stimulation of each of the two known excitatory inputs to these cells: afferents from the medial portion of the dorsolateral thalamus (DLM) and the recurrent axon collateral (LMAN_R) inputs that interconnect neurons within the nucleus (Figure 1C). The DLM synapses are glutamatergic, primarily mediated by AMPA receptors, with a small NMDAR-mediated component evident at the cell's resting potential (V_{REST}) (Livingston

¹Correspondence: cab@phy.ucsf.edu (C.A.B.), ajd@phy.ucsf.edu (A.J.D.)

²Present address: Department of Psychology, University of California, Berkeley, California 94720.

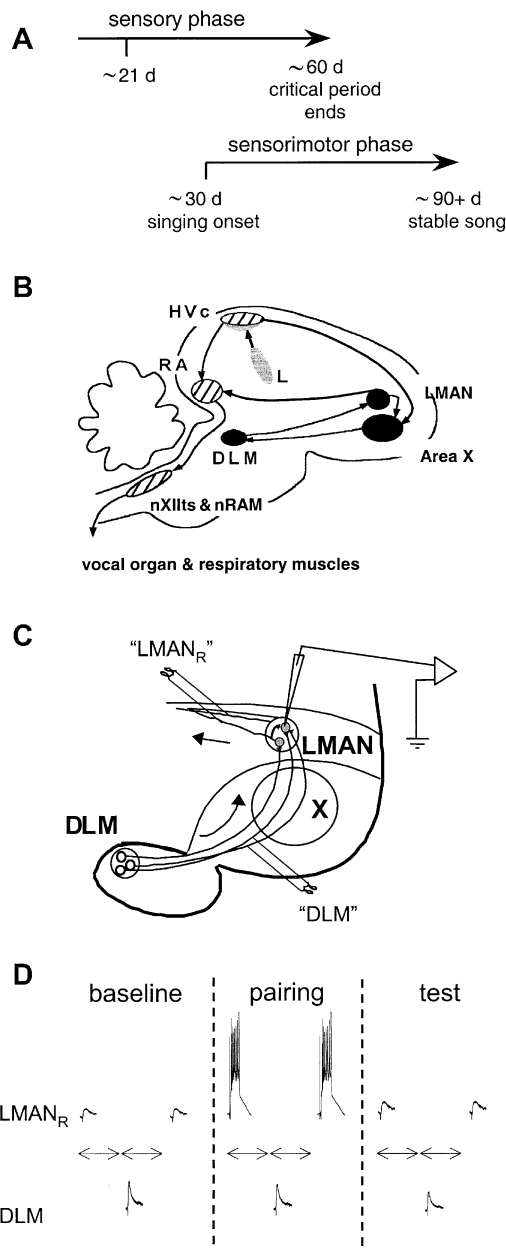


Figure 1. Schematic Representations of Zebra Finch Song Learning, Song System, Slice Preparation, and Plasticity Induction Protocol

(A) Timeline of song learning (days). The sensory phase of learning is restricted to a critical period when birds must hear and memorize a tutor song. During sensorimotor learning, birds listen to their own vocalizations and gradually match them to the memorized tutor song.

(B) Diagram of song system. A parasagittal view of the zebra finch brain showing the motor pathway (hatched) and the anterior fore-brain pathway (black). The Field L complex (L; gray) is the source of auditory input to the song system.

(C) Schematic of recording setup. Slices were cut oblique to the parasagittal plane. The DLM stimulating electrode was placed below Area X to avoid activating LMAN axons projecting to Area X (Nixdorf-Bergweiler et al., 1995a; Vates and Nottebohm, 1995). The LMAN_R stimulating electrode was placed in the LMAN outflow tract, thus activating recurrent axon collaterals. Electrode placement was adjusted such that the neuron being recorded was not antidromically activated.

and Mooney, 1997; Boettiger and Doupe, 1998; Bottjer et al., 1998). The LMAN_R synapses, activated by stimulating the LMAN outflow tract, are also glutamatergic, with a significantly greater NMDAR-mediated component at V_{REST} (20 days) than the DLM synapses (Boettiger and Doupe, 1998).

Pairing Depolarization with LMAN Recurrent Collateral Stimulation at 20 Days Induces Two Forms of Lasting Plasticity

We performed one set of experiments in slices from 20-day-old birds because this age falls within the sensory phase of song learning and precedes the onset of sensorimotor learning (Immelmann, 1969; Eales, 1989) (Figure 1A). To induce plasticity, we repeatedly (40x) delivered single brief (100 ms) pulses of postsynaptic depolarizing current in conjunction with LMAN_R stimulation. Each current injection elicited a burst of approximately 6–10 action potentials. The total duration of the burst approximated the duration of the current pulse. This “pairing” took place over the course of several minutes, during which we continued to stimulate each pathway at low frequency (0.05 Hz, 10 s out of phase) (Figure 1D; see Experimental Procedures for further detail). This pairing protocol produced a long-lasting increase of the LMAN_R excitatory postsynaptic potential (EPSP) slope (Figure 2A). The mean LMAN_R EPSP slope 30 min after pairing onset was increased by $21\% \pm 5\%$ relative to baseline ($n = 12$; $p < 0.001$) (Figure 2B), and when stable recordings could be maintained to 60 min after pairing, EPSP slopes were increased by $33\% \pm 15\%$ relative to baseline ($n = 7$; $p < 0.04$). The changes in the LMAN_R EPSP elicited by the pairing protocol depended on the timing of the first spike elicited by the current injection relative to the onset of the EPSP (see Figure 3). When the first spike occurred after the LMAN_R EPSP onset (“spike lags”), the LMAN_R pathway was potentiated (as in Figure 2). In contrast, when the first spike in the burst preceded the EPSP onset (“spike leads”), potentiation did not occur, and in some cases the LMAN_R EPSP was depressed (Figures 3A and 3B). The pairing protocol induced significantly less potentiation of LMAN_R responses in spike-leading versus spike-lagging experiments ($n = 8$; $p < 0.001$) (Figure 3B).

In the same cells in which LMAN_R pairing potentiated LMAN_R responses, the responses to stimulated DLM inputs were depressed (Figure 2). For these DLM inputs, the postsynaptic depolarization occurred approximately 10 s out of phase with their stimulation, with postsynaptic membrane potential returning to baseline many seconds before DLM EPSPs occurred (Figure 1D). This depression of DLM responses did not depend on whether the pairing induced LMAN_R LTP: unlike the LTP, it was not sensitive to the timing of the action potential (AP) burst relative to the LMAN_R EPSP (spike-lagging versus spike-leading, $p > 0.90$). Because the effect on the DLM

(D) Pairing protocol schematic. Brief (100 ms) postsynaptic depolarizing current was delivered 40x in conjunction with LMAN_R stimulation. Throughout the experiment stimulation of each pathway continued at the baseline frequency (0.05 Hz, 10 s out of phase). For more detail see Experimental Procedures.

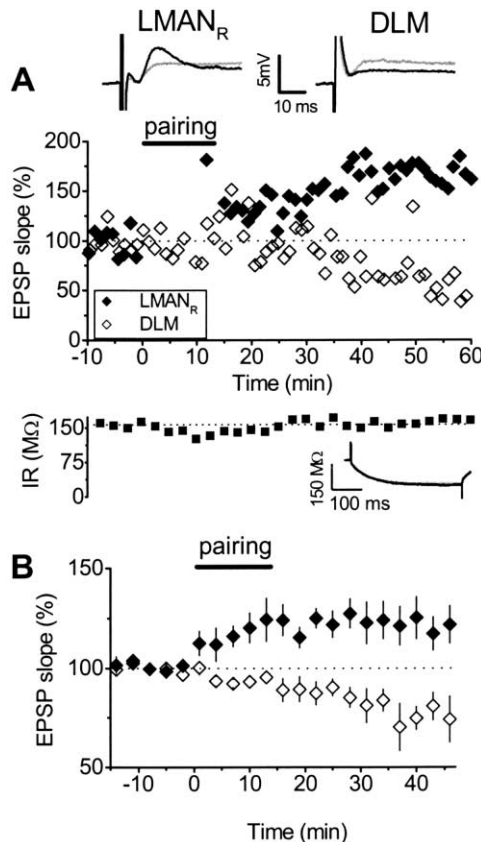


Figure 2. LMAN_R Stimulation Paired with Depolarizing Current Injection Induces Lasting LTP and Concurrent LTD in Slices from Birds in the Early Phase of Sensory Learning

(A) An example of results in a cell from a 22-day-old male zebra finch. Averaged responses to stimulation of each pathway during the baseline period (gray) are shown overlaid with averaged responses 60 min after pairing (black). Experimental time-course showing LMAN_R (◆) and DLM (◇) responses and input resistance (IR; dashed line denotes baseline mean). Inset to IR time-course plot shows response to -100 pA current injection during the baseline period (gray) and 60 min after pairing (black).

(B) Summary data from 12 cells ($n = 12$ birds) with concurrently recorded LMAN_R and DLM responses. The slope of LMAN_R responses at 30 min was significantly increased ($p < 0.001$). DLM responses at 30 min were significantly depressed ($p < 0.02$).

pathway of pairing AP bursts with the LMAN_R pathway did not depend on the relative burst timing, we pooled DLM data from both spike-leading and spike-lagging experiments (Figure 4A). The average depression of DLM EPSPs at 30 min after pairing was $14\% \pm 3\%$ relative to baseline ($n = 20$; $p < 0.001$). In cases where stable recordings were maintained to 60 min after pairing, DLM EPSP slopes were decreased by $30\% \pm 10\%$ ($n = 12$; $p < 0.006$).

Based on the long delay (10 s) between DLM input activation and postsynaptic bursting, it seemed unlikely that synaptic depression was being homosynaptically induced at DLM inputs. Therefore, we performed two control experiments to rule out the possibility that DLM LTD might be due simply to rundown of those inputs as a result of prolonged stimulation. First, we maintained recordings for 30 min while alternately stimulating the

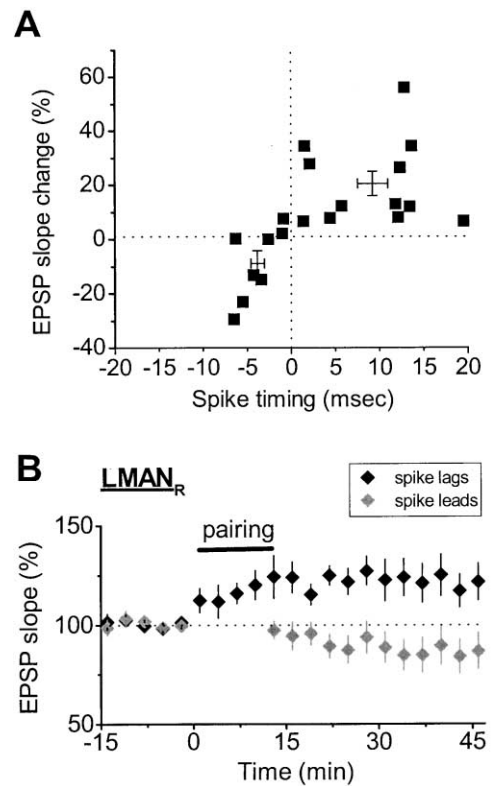


Figure 3. LMAN_R Potentiation Is Dependent on the Relative Timing between the EPSP Onset and the Peak of the First Spike Elicited by the Current Injection

(A) Plot of percent change of LMAN_R EPSP slope at 30 min versus spike timing relative to EPSP onset. Crosshairs denote mean \pm SEM for spike lagging and spike leading data.

(B) Group data comparing effect on LMAN_R EPSPs of spike-lagging (◆, $n = 12$) (see also Figure 2C) versus spike-leading (◇, $n = 8$) pairing. The potentiation elicited by spike-lagging pairing was significantly greater than that induced by spike-leading pairing ($p < 0.001$).

DLM and LMAN_R pathways at the usual low frequency (0.05 Hz). In these experiments, responses remained stable throughout the recording period, as shown in Figure 4B, and demonstrated no significant change from the first 5 min to the final 5 min of recording ($n = 3$; $p > 0.16$). The second experiment was designed to increase the likelihood of DLM fiber rundown; after a stable baseline period, we delivered high-frequency burst stimulation to the DLM pathway (see Experimental Procedures for detail). This manipulation did not significantly change the DLM response strength measured at 30 min ($-1\% \pm 4\%$; $p > 0.87$) or 60 min ($1\% \pm 6\%$; $p > 0.93$) after high-frequency stimulation ($n = 3$) (Figure 4C). The concurrently monitored LMAN_R pathway was also unaffected in these experiments (30 min: $-2\% \pm 4\%$, $p > 0.71$; 60 min: $1\% \pm 6\%$, $p > 0.89$). Because the DLM synaptic responses appeared stable over time in the absence of postsynaptic bursting, we then assessed the effects of repeatedly pairing postsynaptic depolarization of LMAN neurons in close temporal conjunction with stimulation of the DLM pathway, rather than out of phase with this stimulation. When the DLM EPSP occurred within 15 ms before or after the AP burst onset, neither depression

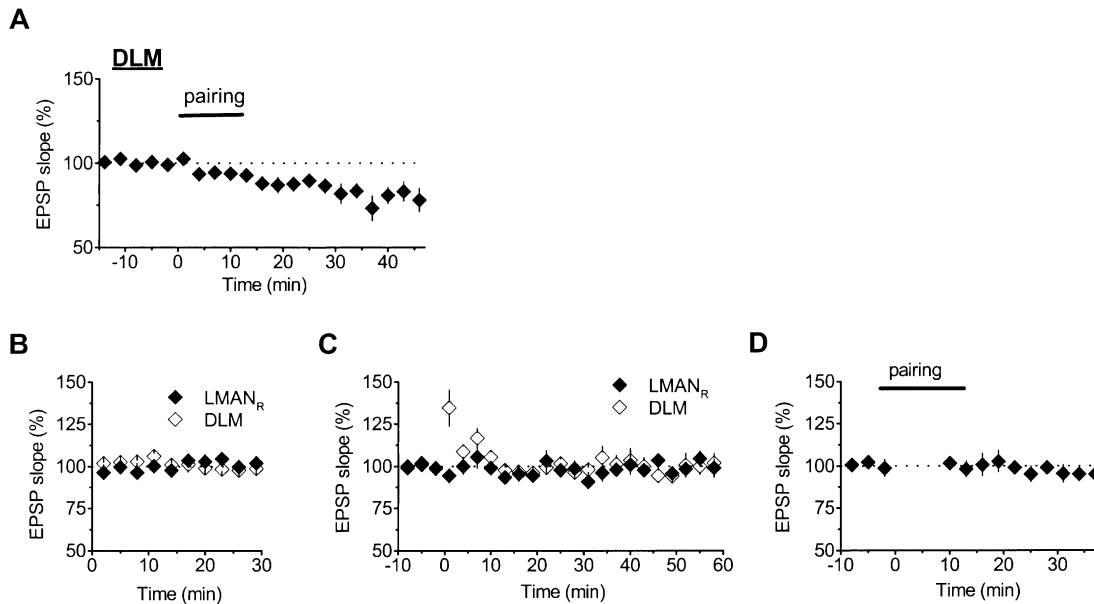


Figure 4. DLM LTD Is Not Due to Rundown

(A) DLM data from LMAN_R pairing experiments, merging spike-lagging ($n = 12$) and spike-leading ($n = 8$) cases. Responses at 30 min were significantly depressed ($p < 0.001$).

(B) Results from experiments ($n = 3$) in which cells were stimulated at baseline frequency (0.05 Hz) for 30 min and no plasticity induction protocol was employed. This resulted in no change to either pathway. Note that error bars smaller than symbol are not visible.

(C) High-frequency stimulation of DLM pathway without pairing protocol. Average of three experiments showing no significant change to either pathway.

(D) Pairing depolarization with DLM pathway stimulation does not induce plasticity. Average of six experiments, no significant change.

nor potentiation resulted ($-2\% \pm 3\%$; $n = 6$; $p > 0.65$) (Figure 4D); there was no significant difference between spike-leading and spike-lagging pairings ($p > 0.79$).

Taken together, these data strengthen the argument that the lasting depression of DLM responses seen after pairing postsynaptic AP bursts with LMAN_R stimulation is an induced plasticity of the synapses rather than simply DLM fiber rundown. Moreover, the long temporal separation between the activity of the presynaptic DLM input and the postsynaptic LMAN neuron suggests that DLM LTD may be induced via a heterosynaptic mechanism. Finally, because depression was not observed when DLM inputs were paired within a narrow time window relative to postsynaptic spiking, the DLM input may be depressed when it is inactive during postsynaptic bursting. Thus, activity of this input simultaneous with postsynaptic bursting may protect the synapse from the otherwise depressing effects of such bursting. LTD exhibiting associativity of this sort could be very useful in the selective pruning of ineffective or unnecessary inputs.

To explore whether these plastic changes in LMAN_R and DLM synapses were presynaptic or postsynaptic in their expression, we examined the effect of our pairing protocol on two parameters traditionally thought to reflect presynaptic release probability (Manabe et al., 1993): the coefficient of variation of response strength (CV) and the paired-pulse ratio (PPR) (see Experimental Procedures). In the LMAN_R pathway, which shows little short-term plasticity at 20 days (Boettiger and Doupe, 1998), neither parameter changed significantly following pairing. Furthermore, the degree of potentiation of LMAN_R responses was not correlated with a change in CV or

PPR ($r^2 = 0.05$ and 0.00 , respectively). In contrast, DLM responses, which typically express strong paired-pulse depression at 20 days (Boettiger and Doupe, 1998), showed significant increases in both CV and PPR ($p < 0.04$ and 0.03 , respectively). In addition, the magnitude of DLM depression was significantly correlated with increases in both CV and PPR ($r^2 = 0.34$, $p < 0.005$ and $r^2 = 0.22$, $p < 0.04$, respectively). These results further highlight the independence of these two forms of plasticity, suggesting that the LMAN_R LTP is due to increased postsynaptic sensitivity, whereas the LTD of the DLM responses is likely due to decreased presynaptic release (Manabe et al., 1993).

LMAN_R Potentiation Is NMDAR Dependent

Given that LMAN_R LTP appeared to be Hebbian and postsynaptically expressed at a synapse with a substantial NMDAR-mediated component (Boettiger and Doupe, 1998), we tested whether LMAN_R LTP depends on NMDAR activation. The presence of $100 \mu\text{M}$ dl-2-amino-5-phosphonovaleric acid (APV) during pairing blocked the increase in LMAN_R responses normally observed at 30 min after spike-lagging pairing and, instead, produced a small but significant depression ($-9\% \pm 5\%$, $n = 10$; $p > 0.05$) (Figure 5A). This result is similar to that recently reported in rat barrel cortex (Feldman, 2000). The reduction in the ability of the pairing protocol to induce LTP when NMDARs were blocked was highly significant ($p < 0.001$) (Figure 5A, open circles). In six cells in which we could repeat the pairing in control ACSF after washing out APV, there was not a significant change in the presence of APV ($-12\% \pm 7\%$; $p > 0.15$), whereas a change of $13\% \pm 3\%$ was obtained after

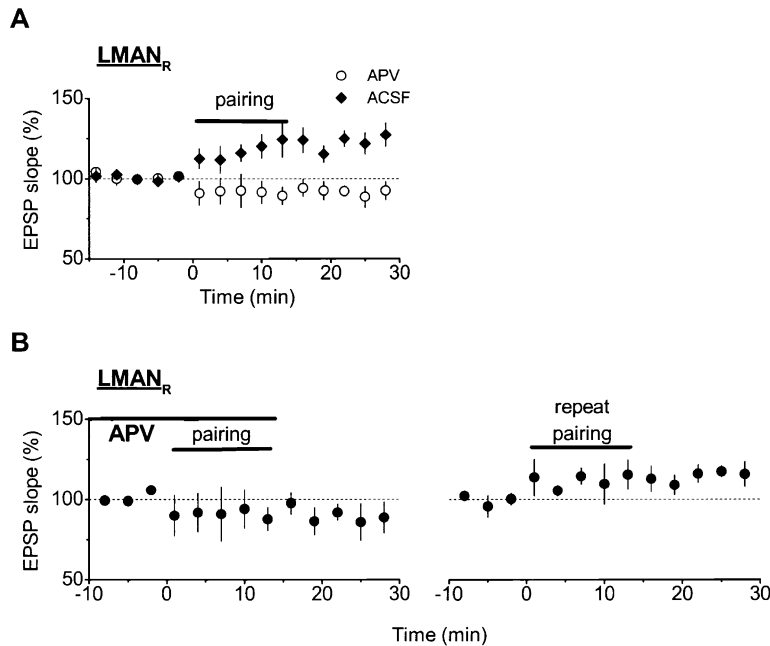


Figure 5. Pairing-Induced LMAN_R Potentiation Is Dependent on NMDAR Activation

(A) Summary plot of ten experiments ($n = 10$ birds) in which LMAN_R pairing was carried out in the presence of APV. LMAN_R APV data (\circ) overlaid with LMAN_R data from experiments in normal ACSF (\bullet) (see also Figure 2C). Pairing in APV completely blocked potentiation of LMAN_R responses, revealing a small depression ($-7\% \pm 4\%$; $p < 0.05$). The ability of the pairing protocol to induce LTP was significantly decreased by the presence of APV ($p < 0.001$).

(B) Group data from six cells where the pairing protocol was repeated after APV was washed out of the recording chamber. Again, the potentiation induced by pairing was reduced significantly in the presence of APV ($p < 0.03$).

washing out APV ($p < 0.004$) (Figure 5B). These data confirmed that pairing induces significantly greater LTP in control ACSF than in APV ($p < 0.03$).

Again in contrast to LMAN_R LTP, the LTD of DLM responses did not appear to depend entirely on NMDAR activation. A small but significant depression of DLM responses at 30 min was still obtained by pairing in conjunction with LMAN_R stimulation in the presence of APV ($-7\% \pm 4\%$, $n = 10$; $p < 0.05$). The depression of DLM responses induced in the presence of APV was not significantly different from that seen in control ACSF ($p > 0.61$).

Both Forms of Plasticity Are Restricted to the Sensory Critical Period

The critical period for sensory learning ends at approximately 60 days in zebra finches, because tutor songs heard after this age are no longer incorporated into the BOS (Immelmann, 1969; Eales, 1985, 1987). Sensory learning not only declines with age but is also impaired by blocking NMDARs in LMAN during tutoring within the critical period (Basham et al., 1996). Whereas synaptic transmission in LMAN may well have been impaired by this manipulation, thalamic input was likely to be largely spared due to its relatively small NMDAR-mediated component (Livingston and Mooney, 1997; Boettiger and Doupe, 1998; Bottjer et al., 1998). LMAN_R LTP, however, would have been prevented by NMDAR blockade, and this could be one factor preventing the incorporation of new tutor song information in this experiment. If so, a decrease in LMAN_R LTP inducibility may also occur developmentally, contributing to the normal closure of the sensory critical period.

To test this prediction, we paired postsynaptic bursts with LMAN_R synaptic stimulation in slices from birds at 60 days of age. The effect of the induction protocol on synaptic inputs of LMAN neurons was strikingly different at this later age. Instead of inducing a potentiation of the LMAN_R responses, a significant depression of LMAN_R

responses was observed at 30 min after spike-lagging pairing ($-14\% \pm 5\%$, $n = 10$; $p < 0.02$) (Figure 6A). This represented a significant decrease in the ability of the pairing protocol to induce LMAN_R LTP at 60 days compared with that at 20 days (20 day data shown for comparison in Figure 6A; $p < 0.001$). In addition, at 60 days, LMAN_R pairing no longer induced LTD of the DLM responses and, indeed, failed to produce a significant change in the DLM responses (mean: $15\% \pm 10\%$, $n = 10$; $p > 0.19$) (Figure 6B). This reflected a significant decrease between 20 and 60 days in the efficacy of the pairing protocol to induce LTD in the DLM pathway ($p < 0.03$; 20 day data included in Figure 6B). Thus, depolarization paired with LMAN_R stimulation no longer induces these two forms of plasticity in LMAN by the end of the sensory acquisition phase of song learning, and instead the same protocol induces depression rather than potentiation at the LMAN_R synapses. Although our results do not rule out the possibility that potentiation of LMAN_R responses and depression of DLM responses could still be induced in older birds by a less physiological protocol, they indicate that the threshold for induction of this plasticity is substantially higher by the end of sensory learning. In addition, the change in sign of the plasticity at the LMAN_R synapses suggests that functionally significant changes have taken place at these connections by 60 days.

LMAN_R LTP in 20-day-old birds was induced by pairing EPSPs with bursts of postsynaptic APs. Plasticity induced in a similar manner has been shown to depend on the synergistic Ca^{2+} signal resulting from the postsynaptic conjunction of EPSPs with backpropagating APs (Magee and Johnston, 1997). Thus, the reduced inducibility of LMAN_R LTP at 60 days could reflect a change in the ability of the protocol to raise postsynaptic Ca^{2+} . For example, if the LMAN_R EPSPs decay more quickly in 60-day-old birds, there may be less postsynaptic Ca^{2+} influx. To begin to examine this possibility, we averaged LMAN_R EPSPs from the baseline period of

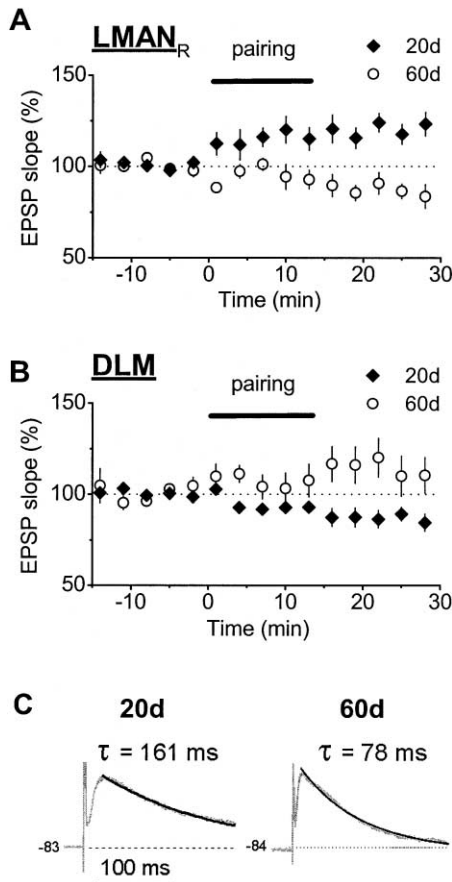


Figure 6. Developmental Changes in LMAN Synaptic Function
(A) Comparison of LMAN_R group data from 20 day (◆) (see also Figure 2C) and 60 day (○) experiments. At 60 days, LMAN_R pairing induced no significant potentiation, instead eliciting depression ($-14\% \pm 5\%$; $p < 0.02$). This represented a significantly different effect of the protocol on LMAN_R responses between 60 and 20 days ($p < 0.001$).
(B) LMAN_R pairing had no significant effect at 60 days (○) on the DLM pathway, eliciting significantly less DLM depression than at 20 days (◆, $p < 0.03$) (see also Figure 2C).
(C) Examples illustrating the developmental decrease in τ_{LR} . LMAN_R EPSPs (gray traces) are shown fit with an exponential decay curve (black). “20d” example from a 23-day-old male; “60d” example from a 67-day-old male. Note that whereas the 60 day EPSP has fully decayed to baseline by 200 ms after stimulation, the 20 day EPSP has only decayed by $\sim 75\%$. EPSPs scaled to same amplitude.

pairing experiments, fit them with single exponential decays to estimate the synaptic time constants (τ_{LR}), and compared the τ_{LR} values in the two age groups (example traces shown in Figure 6C; see Experimental Procedures for more detail). We found that at 20 days τ_{LR} was significantly longer (169 ± 16 ms) than at 60 days (60 ± 12 ms; $p < 0.001$), indicating that LMAN_R EPSPs decay more slowly at 20 days than at 60 days. However, EPSP decay time depends both on the decay of the underlying synaptic currents and on the cell's membrane time constant (τ_{MEM}). Thus, a component of τ_{LR} shortening between 20 and 60 days is likely due to the decreased membrane capacitance of LMAN cells caused by the dramatic reduction in surface area of these cells over this time period (Nixdorf-Bergweiler et al., 1995b), which should result in a shortened mem-

brane time constant (Johnston and Wu, 1995). We measured τ_{MEM} directly and found that it indeed decreased nearly 2-fold from 20 to 60 days (49 ± 3 ms versus 25 ± 5 ms; $p < 0.001$). To assess whether age differences in τ_{LR} exist beyond that attributable to the age-dependent difference in τ_{MEM} , we applied an ANCOVA to τ_{LR} with τ_{MEM} as a covariate. The ANCOVA indicated that the decrease in τ_{LR} exhibited by 60-day-old animals remained significant, even after accounting for the variance associated with τ_{MEM} ($p < 0.05$; slope of the 20 and 60 day regressions of τ_{LR} versus τ_{MEM} was not significantly different, $p > 0.99$). These findings indicate that τ_{MEM} can account for only some of the decrease in τ_{LR} with age. In the same cells in which we measured τ_{LR} , the decay time constant of DLM EPSPs (τ_{DLM}) also decreased from 20 to 60 days (91 ± 11 ms versus 59 ± 9 ms; $p < 0.05$). However, an ANCOVA indicated no developmental decrease in τ_{DLM} after factoring out τ_{MEM} ($p > 0.46$). Besides τ_{MEM} , another major determinant of the time course of EPSP decay is the time course of the underlying synaptic current. Thus, our results provide indirect evidence consistent with a developmental shortening of LMAN_R synaptic currents. A potential caveat is that these experiments were performed with inhibitory transmission intact. Thus, a developmental increase in polysynaptic IPSPs could account for the decrease we saw in EPSP decay times in LMAN. However, our data from the DLM synapse argue against this possibility because the decrease we describe does not exceed those reported in developmental studies of the DLM synaptic currents with IPSPs blocked (Livingston and Mooney, 1997; Livingston et al., 2000).

Discussion

The results presented here demonstrate that long-term synaptic plasticity can be induced in a nucleus required for song learning by pairing recurrent collateral EPSPs with postsynaptic bursts of APs. The induced plasticity consisted of an LTP of intrinsic LMAN synapses and a concurrent LTD of thalamic input synapses. The LMAN_R LTP was dependent on both NMDAR activation and the timing of the AP burst, whereas the DLM LTD depended on neither. Both LMAN_R LTP and DLM LTD were present at a time when sensory learning was occurring and were no longer evident by the close of the sensory critical period. This timing suggests that each may play a role in tutor song memorization as well as in the early stages of sensorimotor evaluation and refinement of song.

Hebbian LTP at Recurrent Synapses

LMAN_R LTP is similar to previously described forms of cortical LTP in its magnitude, rise time, NMDAR dependence, and developmental restriction. These properties are consistent with the cortical-like cellular physiology of LMAN principal cells (Livingston and Mooney, 1997; Boettiger and Doupe, 1998) and the frontal-cortex-like connectivity of LMAN (Bottjer and Johnson, 1997). LMAN_R LTP also exhibits timing dependence, a computationally important feature (Roberts, 1999; Song et al., 2000) recently described in several systems (Bell et al., 1997; Magee and Johnston, 1997; Markram et al., 1997; Bi and Poo, 1998; Debanne et al., 1998; Zhang et al., 1998; Egger et al., 1999; Feldman, 2000). Although the

EPSP-AP burst pairing used in the present study establishes the timing dependence of the LMAN_R LTP, further experiments using a single AP may provide a more complete description of the timing rule for LMAN_R synapses. Moreover, the lack of LTP induction when the first spike of a burst preceded the EPSP, despite subsequent spikes following the EPSP, suggests that the first spike plays a critical role in determining the sign of long-term plasticity (see also Zhang et al., 1998).

Timing-dependent LMAN_R plasticity, coupled with DLM LTD, provides a simple and plausible mechanism for storage and recognition of a temporal pattern, an idea that has been theoretically explored (Gerstner et al., 1993). According to this line of thinking, LMAN_R LTP could contribute to the developmental emergence of LMAN neurons strongly selective for particular temporal combinations of sound (Doupe, 1997). Two pieces of evidence lend support to this hypothesis. First, the intrinsic circuitry of LMAN was recently shown to amplify selective responses of adult LMAN neurons (Rosen and Mooney, 2000). Second, effective tutor memorization depends on normal NMDAR function in LMAN (Basham et al., 1996). Our present results suggest that, during sensory learning, one such NMDAR function may be the enabling of Hebbian synaptic plasticity at LMAN_R connections, supporting the hypothesis that excitatory feedback connections are key sites of synaptic plasticity within neural networks (Hua et al., 1999). Furthermore, the similarity of LMAN plasticity to that in developing sensory cortex provides further support for the idea that shared cellular mechanisms underlie experience-dependent development and learning (Carew et al., 1998).

Developmental Restriction of LMAN_R LTP

Whereas a number of mechanisms may contribute to the developmental restriction of LMAN_R LTP, the fact that age and APV have the same effect (i.e., changing the sign of the plasticity) points to a change in NMDAR function at these synapses from 20 to 60 days. Specifically, one would predict a change resulting in reduced postsynaptic Ca²⁺ influx, thereby impairing the synergistic Ca²⁺ signal generated by the conjunction of an EPSP and a back-propagating AP (Magee and Johnston, 1997). Such reduction of postsynaptic Ca²⁺ influx is indeed accomplished in some systems by a developmental shortening of NMDAR-mediated currents (Carmignoto and Vicini, 1992; Hestrin, 1992; Crair and Malenka, 1995), a mechanism consistent with our results showing a shortening of LMAN_R EPSPs between 20 and 60 days. However, our results do not distinguish between a decrease in the NMDA to AMPA ratio at the LMAN_R synapses (Crair and Malenka, 1995; Stark and Perkel, 1999), and a decrease in the contribution of the slow NMDAR-2B subunit (NR2B) (Monyer et al., 1992). Future studies using the whole-cell patch technique, rather than sharp electrodes, should determine whether a change in the NMDA to AMPA ratio occurs developmentally at LMAN_R synapses. Interpreting developmental changes in LMAN_R current kinetics will be difficult, however, given the poor space clamp attainable in LMAN neurons (Livingston and Mooney, 1997) and the large developmental decrease in τ_{MEM} that we describe here. Anatomical studies, on the other hand, show a developmental decrease in NR2B expression in LMAN (Basham

et al., 1999; Singh et al., 2001). Although this decline could reflect both LMAN_R and DLM synapses, our synaptic decay results at both synapses, coupled with recent studies of the DLM synapses (Livingston et al., 2000), suggest that the change in NR2B expression in LMAN includes substantial change at the LMAN_R synapses. Therefore, although it has been suggested that song learning does not require slow NMDAR currents in LMAN, on the basis of studies of the DLM inputs (Livingston et al., 2000), our results raise the alternate possibility that slowly decaying currents at LMAN_R synapses are found only prior to the close of the sensory learning phase and, thus, may indeed play a critical role in learning by enabling LMAN_R LTP (Tang et al., 1999).

LTD of Thalamic Afferent Input

The protocol that induces LMAN_R LTP causes a concurrent LTD of the DLM inputs. This LTD is independent of the LTP, however, because manipulations that prevent LMAN_R LTP at 20 days (APV application, shifting the relative timing of the current injection) do not eliminate DLM LTD. Whereas the present results do not rule out a homosynaptic induction mechanism for DLM LTD, the long delay between the postsynaptic voltage effects and DLM input activation suggests the likelihood of a heterosynaptic mechanism. Heterosynaptic LTD has been described in the hippocampus (Scahill et al., 1996), and although DLM LTD is similar in magnitude, it differs in other respects. First, DLM LTD does not appear to share with hippocampal heterosynaptic LTD a complete dependence on NMDARs. Second, DLM LTD appears to be expressed presynaptically, as a decreased probability of release, on the basis of the correlation of the depression with increases in the PPR and CV. Although short-term heterosynaptic depression expressed presynaptically has been described (Weisskopf et al., 1993; Dittman and Regehr, 1997; Vogt and Nicoll, 1999; Ohno-Shosaku et al., 2000; Parker, 2000; Wilson and Nicoll, 2001), previous findings have not included long-lasting forms of such depression induced by such means. Thus, DLM LTD may represent another form of long-term plasticity.

Strikingly, when the DLM input is stimulated within a very narrow temporal window relative to postsynaptic bursting, the LTD seems to be prevented. Activity of this input simultaneous with postsynaptic bursting therefore appears to "protect" the synapse against the otherwise depressing effects of such bursting. In vivo, such a property would confer a mechanism for activity-dependent synaptic competition: synaptic inputs that are inactive during postsynaptic bursting and, thus, not likely to have contributed to such bursting, would be weakened. The utility of this mechanism is such that LTD of this type may well prove to be a general feature of thalamic input synapses.

Although the underlying mechanism is unresolved, we speculate that the postsynaptic burst firing used in our induction protocol could cause both peptide release and glutamate spillover from LMAN_R terminals, potentially acting on DLM terminals to depress release. A candidate peptide is calcitonin gene-related peptide, which is expressed in LMAN principal neurons (Bottjer et al., 1997) and was recently shown to presynaptically depress release at the lamprey reticulospinal synapses (Parker,

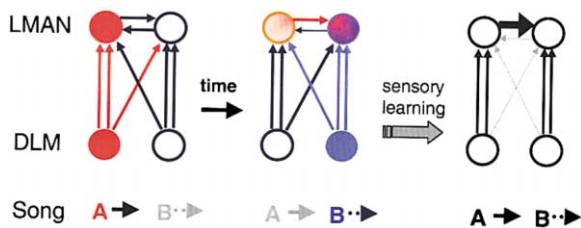


Figure 7. A Simple Model Depicting a Possible Role for LMAN Plasticity in Song Learning

During sensory learning, LMAN plasticity may establish a prediction within the recurrent circuitry of the temporal pattern of DLM afferent activation elicited by tutor song. A segment of tutor song “A” would elicit firing in a subset of DLM projection neurons (left panel, shown in red), which in turn would activate a subset of LMAN neurons (also in red), including their recurrent projections onto other LMAN neurons. Those LMAN neurons (shown in blue and red) activated by collateral inputs and simultaneously by DLM inputs responding to the next chunk of song “B” (middle panel, shown in blue) would experience the conjunction required for LMAN_R LTP. Over the course of sensory learning, the spike-timing dependent strengthening of LMAN_R synapses would come to reflect the temporal pattern of DLM afferent activation by tutor song (right panel). Moreover, DLM inputs not participating in driving particular LMAN neurons to fire would weaken due to LTD (dotted lines), thus refining the DLM to LMAN projection.

2000). Alternatively, the postsynaptic depolarization may cause the dendritic release of a retrograde messenger, such as seen in depolarization-induced suppression of inhibition or excitation (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Elucidation of the underlying mechanism of DLM LTD should also provide insight into the cause of its developmental restriction. This restriction suggests a role for DLM LTD in the synaptic pruning of the DLM afferent projection, which occurs during the period of development studied here (Johnson and Bottjer, 1992; Iyengar et al., 1999).

Implications for Plasticity in LMAN During Sensory Learning: A Simple Model

Our results demonstrate that, during the sensory critical period, pairing synaptic activation with postsynaptic bursting induces a lasting potentiation at recurrent excitatory synapses and a concomitant lasting depression in the thalamic input synapses in a telencephalic structure. This plasticity could be useful for generating connectivity within LMAN that reflects the temporal pattern of DLM afferent activity elicited by the tutor song and, thus, can predict that pattern. That is, if different subsets of DLM afferents fire at different time points in response to the sound of the tutor song, LMAN_R LTP would cause LMAN neurons activated by DLM at one point in time to strengthen their connections onto LMAN neurons activated by DLM at a subsequent time point (Figure 7). In contrast, because of the spike timing dependence of LMAN_R LTP, the reciprocal LMAN_R connections would weaken or remain static. In addition, DLM afferent LTD would refine the projection of DLM inputs, weakening connections that do not participate in driving LMAN neurons to fire. These changes in synaptic strength over the course of sensory learning would come to represent the temporal pattern of DLM afferent activation in response to tutor song. Circuitry organized in this fashion

could represent a memory of the tutor song, reminiscent of a proposed model for sequence prediction in the hippocampus (Abbott and Blum, 1996). During sensorimotor learning, such circuitry could then preferentially reinforce motor sequences produced by the bird that sound adequately similar to the tutor song (Troyer and Doupe, 2000). It is also possible that these forms of plasticity are important to the early sensorimotor phase of song learning, allowing singing experience to rapidly shape the network in LMAN (Solis and Doupe, 1999, 2000). In conclusion, our results indicate that the circuitry in LMAN may be a particularly advantageous site for pursuing a causal link between experience-dependent changes in synaptic strength and the learning of a complex behavior.

Experimental Procedures

Slice Preparation and Recording Techniques

Parasagittal slices 400 μ m in thickness were prepared from the anterior forebrain of male zebra finches as previously described (Boettiger and Doupe, 1998). All procedures were done in accordance with protocols approved by the UCSF animal care and use committee. Two age groups were used: \sim 3 weeks posthatch (20 days; mean: 23 ± 2 days [SD]; range: 18–26; $n = 37$), and \sim 9 weeks posthatch (60 days; mean: 62 ± 3 days [SD]; range: 57–67; $n = 10$). Slices were transferred to a recording chamber at room temperature, perfused at a rate of 2 ml/min with a solution consisting of 134 mM NaCl, 3 mM KCl, 1.1 mM NaH_2PO_4 , 1.3 mM MgSO_4 , 2.4 mM CaCl_2 , 25.7 mM NaHCO_3 , and 12 mM dextrose, and bubbled with 95% O_2 /5% CO_2 . Intracellular electrode tips were filled with 2% Biocytin in 2 M potassium acetate (pH 7.2), and the remainder of the electrode was filled with 3 M potassium acetate. Chemicals were obtained from Sigma or Fisher.

After at least 1 hr of recovery time, penetrations were made “blind” within the readily visible borders of LMAN. Penetrations that met the following criteria were maintained: (1) resting membrane potential (V_{REST}) ≤ -50 mV (2) input resistance ≥ 20 M Ω , (3) threshold current to elicit an action potential < 1 nA, and (4) action potentials overshoot 0 mV. Input pathways were activated using monopolar current pulses (50–100 μ s). In each experiment, one stimulating electrode (FHC, Brunswick, ME) was placed ventro-caudal to Area X, activating DLM axons, and a second was placed in the outflow tract of LMAN, activating recurrent axon collaterals (Figure 1C). To examine monosynaptic responses, we selected for analysis those EPSPs that showed a fixed latency with increasing current intensity and a stable amplitude in response to stimulation at 1 Hz. The current intensity was set for each stimulating electrode to minimize both response failures as well as polysynaptic recruitment. During the baseline period, test stimuli were delivered to each pathway (in alternation) at 0.05 Hz. During the pairing procedure, the 0.05 Hz stimulation rate was maintained for both inputs, and a 100 ms depolarizing current injection (~ 1 nA) was paired 40 times with the LMAN_R input. This protocol was chosen because similar protocols effectively elicit plasticity in other systems (Gustafsson et al., 1987; Huang and Kandel, 1998; Buonomano, 1999), and it has the advantage of physiological plausibility, mimicking natural bursting conditions in the nucleus. Test stimuli were either single pulse or paired pulses at short intervals. DLM high-frequency burst protocol consisted of five trains (intertrain interval: 20 s) of 10 bursts each (100 Hz, 40 ms duration, 200 ms interburst interval). Recordings were made with an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA) in “bridge” mode, filtered at 3 kHz. The amplified signal was digitized at 10 kHz and analyzed off-line using the DataWave Experimenter’s Workbench hardware and software package (DataWave Technologies, Longmont, CO). Cell input resistance and τ_{MEM} were measured from hyperpolarizing current injections (400 ms; 50–100 pA) made throughout the duration of experiments. EPSP traces and cell input resistance were monitored on-line throughout each experiment. Spike-lagging experiments (at 20 days) were interleaved with spike-leading, APV, high-frequency DLM stimulation, 30 min no stimula-

tion, and 60 day experiments over the course of these studies (and in some cases on the same day with different slices).

Data Analysis and Statistics

Experiments were excluded from analysis if EPSP slope values for both pathways did not exhibit ≥ 10 min stable baseline period, if the cell's input resistance changed by more than 15%, or if the stimulation artifacts noticeably changed size or shape. We also excluded cases where synaptic stimulation elicited action potentials following the pairing procedure, due to our resulting inability to measure EPSP slopes uncontaminated by spikes. These cases likely reflect substantial potentiation of responses; thus, our results may underestimate the true effect of our protocol. To minimize the contribution of polysynaptic responses and voltage-gated conductances, all analyses of synaptic strength were based on the slope of the initial rising phase of the EPSP. In sweeps where slope measurements were contaminated by the depolarization during the pairing procedure (all spike-leading experiments and five spike-lagging experiments), the slope measurement during pairing was not included in the group data time-course plots. PPRs were calculated as EPSP 2/EPSP 1, using EPSP slopes, when pairs of stimuli were delivered 50 ms apart. CV was calculated as the standard deviation of the slope divided by the mean EPSP slope. For group data time-course plots, data from each cell were aligned with respect to the onset of the pairing protocol ($t = 0$), normalized to the average value from the 10 min period immediately preceding the pairing protocol ("baseline"), distributed into 3 min bins, and then averages were generated from all cells. Averaged values are given as mean \pm SEM. To estimate synaptic τ (τ_{LR} or τ_{DLM}), we generated EPSP averages and then performed a least-squares fit of the falling phase of each averaged trace with a single exponential decay function, with the asymptote constrained to the baseline value (Origin 5.1, Microcal Software, Northampton, MA). We made every effort to restrict our decay fits to the monosynaptic component of the response, including the rejection of traces with obvious polysynaptic contamination from averages and, when necessary, restricting the curve-fitting to the uncontaminated portion of the averaged trace. Statistical comparisons were performed on normalized data using t test. For ANCOVA, we compared the two age groups, with τ_{LR} or τ_{DLM} as the dependent variable and τ_{MEM} as the concomitant variable. Criterion for significance in all tests was $p < 0.05$.

Acknowledgments

We thank L. Abbott, M. Brainard, D. Buonomano, N. Hessler, G. Hjelmstad, R. Nicoll, M. Solis, and M. Stryker for useful comments on the manuscript. We also thank A. Arteseros for valuable technical assistance and N. Molyneux for administrative support. This work was supported by NIH grants MH5937 and NS34835, the John Merck fund (to A.J.D.), and predoctoral NRSA MH11896 (to C.A.B.).

Received October 10, 2000; revised June 21, 2001.

References

- Aamodt, S.M., Kozlowski, M.R., Nordeen, E.J., and Nordeen, K.W. (1992). Distribution and developmental change in [3H]MK-801 binding within zebra finch song nuclei. *J. Neurobiol.* 23, 997–1005.
- Abbott, L.F., and Blum, K.I. (1996). Functional significance of long-term potentiation for sequence learning and prediction. *Cereb. Cortex* 6, 416–416.
- Basham, M.E., Sohrabji, F., Singh, T.D., Nordeen, E.J., and Nordeen, K.W. (1999). Developmental regulation of NMDA receptor 2B subunit mRNA and ifenprodil binding in the zebra finch anterior forebrain. *J. Neurobiol.* 39, 155–167.
- Basham, M.E., Nordeen, E.J., and Nordeen, K.W. (1996). Blockade of NMDA receptors in the anterior forebrain impairs sensory acquisition in the zebra finch. *Neurobiol. Learn. Mem.* 66, 295–304.
- Bell, C.C., Han, V.Z., Sugawara, Y., and Grant, K. (1997). Synaptic plasticity in a cerebellum-like structure depends on spike timing, synaptic strength and cell type. *Nature* 387, 278–281.
- Bi, G., and Poo, M.-M. (1998). Synaptic modifications in cultured

- hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J. Neurosci.* 18, 10464–10472.
- Boettiger, C.A., and Doupe, A.J. (1998). Intrinsic and thalamic excitatory inputs onto songbird LMAN neurons differ in their pharmacological and temporal properties. *J. Neurophysiol.* 79, 2615–2628.
- Bottjer, S.W., Brady, J.D., and Walsh, J.P. (1998). Intrinsic and synaptic properties of neurons in the vocal-control nucleus IMAN from in vitro slice preparations of juvenile and adult zebra finches. *J. Neurobiol.* 37, 642–658.
- Bottjer, S.W., Halsema, K.A., Brown, S.A., and Miesner, E.A. (1989). Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches. *J. Comp. Neurol.* 279, 312–326.
- Bottjer, S.W., and Johnson, F. (1997). Circuits, hormones, and learning: vocal behavior in songbirds. *J. Neurobiol.* 33, 602–618.
- Bottjer, S.W., Miesner, E.A., and Arnold, A.P. (1984). Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science* 224, 901–903.
- Bottjer, S.W., Roselinsky, H., and Tran, N.B. (1997). Sex differences in neuropeptide staining of song-control nuclei in zebra finch brains. *Brain Behav. Evol.* 50, 284–303.
- Brainard, M.S., and Doupe, A.J. (2000). Interruption of a basal ganglia-forebrain circuit prevents plasticity of learned vocalizations. *Nature* 404, 762–766.
- Buonomano, D.V. (1999). Distinct functional types of associative long-term potentiation in neocortical and hippocampal pyramidal neurons. *J. Neurosci.* 19, 6748–6754.
- Carew, T.J., Menzel, R., and Shatz, C.J., eds. (1998). *Mechanistic Relationships between Development and Learning: Beyond Metaphor*. (New York: John Wiley & Sons).
- Carmignoto, G., and Vicini, S. (1992). Activity-dependent decrease in NMDA receptor responses during development of the visual cortex. *Science* 258, 1007–1011.
- Crair, M.R., and Malenka, R.C. (1995). A critical period for long-term potentiation at thalamocortical synapses. *Nature* 375, 325–328.
- Debanne, D., Gähwiler, B.H., and Thompson, S.M. (1998). Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampus slice cultures. *J. Physiol.* 507, 237–247.
- Dittman, J.S., and Regehr, W.G. (1997). Mechanism and kinetics of heterosynaptic depression at a cerebellar synapse. *J. Neurosci.* 17, 9048–9059.
- Doupe, A.J. (1997). Song- and order-selective neurons in the songbird anterior forebrain and their emergence during vocal development. *J. Neurosci.* 17, 1147–1167.
- Eales, L.A. (1985). Song learning in zebra finches: some effects of song model availability on what is learnt and when. *Anim. Behav.* 33, 1293–1300.
- Eales, L.A. (1987). Song learning in female-raised zebra finches: another look at the sensitive phase. *Anim. Behav.* 35, 1356–1365.
- Eales, L.A. (1989). The influences of visual and vocal interaction on song learning in zebra finches. *Anim. Behav.* 37, 507–508.
- Egger, V., Feldmeyer, D., and Sakmann, B. (1999). Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. *Nat. Neurosci.* 2, 1098–1105.
- Feldman, D.E. (2000). Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. *Neuron* 27, 45–56.
- Gerstner, W., Ritz, R., and van Hemmen, J.L. (1993). Why spikes? Hebbian learning and retrieval of time-resolved excitation patterns. *Biol. Cybern.* 69, 503–515.
- Gustafsson, B., Wigström, H., Abraham, W.C., and Huang, Y.Y. (1987). Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *J. Neurosci.* 7, 774–780.
- Hebb, D.O. (1949). *The Organization of Behavior*. (New York: John Wiley & Sons).
- Hensch, T.K., Gordon, J.A., Brandon, E.P., McKnight, G.S., Idzerda, R.L., and Stryker, M.P. (1998). Comparison of plasticity in vivo and in vitro in the developing visual cortex of normal and protein kinase A RI β -deficient mice. *J. Neurosci.* 18, 2108–2117.

- Hestrin, S. (1992). Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. *Nature* 357, 686–689.
- Hua, S.E., Houk, J.C., and Mussa-Ivaldi, F.A. (1999). Emergence of symmetric, modular, and reciprocal connections in recurrent networks with Hebbian learning. *Biol. Cybern.* 81, 211–225.
- Huang, Y.Y., and Kandel, E.R. (1998). Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. *Neuron* 21, 169–178.
- Immelmann, K. (1969). Song development in the zebra finch and other estrildid finches. In *Bird Vocalizations*, R.A. Hinde, ed. (London: Cambridge University Press), pp. 64–71.
- Iyengar, S., Viswanathan, S.S., and Bottjer, S.W. (1999). Development of topography within song control circuitry of zebra finches during the sensitive period for song learning. *J. Neurosci.* 19, 6037–6057.
- Johnson, F.W., and Bottjer, S.W. (1992). Growth and regression of thalamic efferents in the song-control system of male zebra finches. *J. Comp. Neurol.* 326, 442–450.
- Johnston, D., and Wu, S.M.-S. (1995). *Foundations of Cellular Neurophysiology*. (Cambridge: MIT Press).
- Kirkwood, A., Lee, H.-K., and Bear, M.F. (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature* 375, 328–331.
- Kreitzer, A.C., and Regehr, W.G. (2001). Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29, 717–727.
- Livingston, F.S., and Mooney, R. (1997). Development of intrinsic and synaptic properties in a forebrain nucleus essential to avian song learning. *J. Neurosci.* 17, 8997–9009.
- Livingston, F.S., White, S.A., and Mooney, R. (2000). Slow NMDA-EPSCs at synapses critical for song development are not required for song learning in zebra finches. *Nat. Neurosci.* 3, 482–488.
- Magee, J.C., and Johnston, D. (1997). A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* 275, 209–213.
- Manabe, T., Wyllie, D.J.A., Perkel, D.J., and Nicoll, R.A. (1993). Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *J. Neurophysiol.* 70, 1451–1459.
- Markram, H., Lübke, J., Frotscher, M., and Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* 275, 213–215.
- Martin, S.J., Grimwood, P.D., and Morris, R.G.M. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. *Ann. Rev. Neurosci.* 23, 649–711.
- Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., and Seeburg, P.H. (1992). Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* 256, 1217–1221.
- Mooney, R., and Konishi, M. (1991). Two distinct inputs to an avian song nucleus activate different glutamate receptor subtypes on individual neurons. *Proc. Natl. Acad. Sci. USA* 88, 4075–4079.
- Morrison, R., and Nottebohm, F. (1993). Role of a telencephalic nucleus in the delayed song learning of socially isolated zebra finches. *J. Neurobiol.* 24, 1045–1064.
- Nixdorf-Bergweiler, B.E., Lips, M.B., and Heinemann, U. (1995a). Electrophysiological and morphological evidence for a new projection of LMAN-neurons towards area X. *Neuroreport* 6, 1729–1732.
- Nixdorf-Bergweiler, B.E., Wallhäusser-Franke, E., and DeVoogd, T.J. (1995b). Regressive development in neuronal structure during song learning in birds. *J. Neurobiol.* 27, 204–215.
- Nordeen, K.W., and Nordeen, E.J. (1992). Auditory feedback is necessary for the maintenance of stereotyped song in adult zebra finches. *Behav. Neural. Biol.* 57, 58–66.
- Nottebohm, F., Kelley, D.B., and Paton, J.A. (1982). Connections of vocal control nuclei in the canary telencephalon. *J. Comp. Neurol.* 207, 344–357.
- Nottebohm, F., Stokes, T.M., and Leonard, C.M. (1976). Central control of song in the canary, *Serinus canarius*. *J. Comp. Neurol.* 165, 457–486.
- Ohno-Shosaku, T., Sawada, S., and Kano, M. (2000). Heterosynaptic expression of depolarization-induced suppression of inhibition (DSI) in rat hippocampal cultures. *Neurosci. Res.* 36, 67–71.
- Ohno-Shosaku, T., Maejima, T., and Kano, M. (2001). Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29, 729–738.
- Okuhata, S., and Saito, N. (1987). Synaptic connections of thalamo-cerebral vocal control nuclei of the canary. *Brain Res. Bull.* 18, 35–44.
- Parker, D. (2000). Presynaptic and interactive peptidergic modulation of reticulospinal synaptic inputs in the lamprey. *J. Neurophysiol.* 83, 2497–2507.
- Roberts, P.D. (1999). Computational consequences of temporally asymmetric learning rules: I. Differential Hebbian learning. *J. Comput. Neurosci.* 7, 235–246.
- Rosen, M.J., and Mooney, R. (2000). Intrinsic and extrinsic contributions to auditory selectivity in a song nucleus critical for vocal plasticity. *J. Neurosci.* 20, 5437–5448.
- Scanziani, M., Malenka, R.C., and Nicoll, R.A. (1996). Role of intercellular interactions in heterosynaptic long-term depression. *Nature* 380, 446–450.
- Scharff, C., and Nottebohm, F. (1991). A comparative study of the behavioral deficits following lesions of the various parts of the zebra finch song system: implications for vocal learning. *J. Neurosci.* 11, 2896–2913.
- Singh, T.D., Basham, M.E., Nordeen, E.J., and Nordeen, K.W. (2000). Early sensory and hormonal experience modulate age-related changes in NR2B mRNA within a forebrain region controlling avian vocal learning. *J. Neurobiol.* 44, 82–94.
- Sohrabji, F., Nordeen, E.J., and Nordeen, K.W. (1990). Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. *Behav. Neural. Biol.* 53, 51–63.
- Solis, M.M., and Doupe, A.J. (1999). Contributions of tutor and bird's own song experience to neural selectivity in the songbird anterior forebrain. *J. Neurosci.* 19, 4559–4584.
- Solis, M.M., and Doupe, A.J. (2000). Compromised neural selectivity for song in birds with impaired sensorimotor learning. *Neuron* 25, 109–121.
- Song, S., Miller, K.D., and Abbott, L.F. (2000). Competitive Hebbian learning through spike-timing-dependent synaptic plasticity. *Nat. Neurosci.* 3, 919–923.
- Stark, L.L., and Perkel, D.J. (1999). Two-stage, input-specific synaptic maturation in a nucleus essential for vocal production in the zebra finch. *J. Neurosci.* 19, 9107–9116.
- Tang, Y.-P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., and Tsien, J.Z. (1999). Genetic enhancement of learning and memory in mice. *Nature* 401, 63–69.
- Troyer, T.W., and Doupe, A.J. (2000). An associational model of birdsong sensorimotor learning. II. Temporal hierarchies and the learning of song sequence. *J. Neurophysiol.* 84, 1224–1239.
- Vates, E.G., and Nottebohm, F. (1995). Feedback circuitry within a song-learning pathway. *Proc. Natl. Acad. Sci. USA* 92, 5139–5143.
- Vogt, K.E., and Nicoll, R.A. (1999). Glutamate and gamma-aminobutyric acid mediate a heterosynaptic depression at mossy fiber synapses in the hippocampus. *Proc. Natl. Acad. Sci. USA* 96, 1118–1122.
- Weisskopf, M.G., Zalutsky, R.A., and Nicoll, R.A. (1993). The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. *Nature* 362, 423–427.
- Williams, H., and Mehta, N. (1999). Changes in adult zebra finch song require a forebrain nucleus that is not necessary for song production. *J. Neurobiol.* 39, 14–28.
- Wilson, R.I., and Nicoll, R.A. (2001). Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature* 410, 588–592.
- Zhang, L.I., Tao, H.W., Holt, C.E., Harris, W.A., and Poo, M.-M. (1998). A critical window for cooperation and competition among developing retinotectal synapses. *Nature* 395, 37–44.