Synaptic and Molecular Mechanisms Regulating Plasticity during Early Learning

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ABSTRACT: Many behaviors are learned most easily during a discrete developmental period, and it is generally agreed that these "sensitive periods" for learning reflect the developmental regulation of molecular or synaptic properties that underlie experience-dependent changes in neural organization and function. Avian song learning provides one example of such temporally restricted learning, and several features of this behavior and its underlying neural circuitry make it a powerful model for studying how early experience sculpts neural and behavioral organization. Here we describe evidence that within the basal ganglia–thalamocortical loop implicated in vocal learning, song acquisition engages *N*-methyl-D-aspartate receptors (NMDARs), as well as signal transduction cascades strongly implicated in other instances of learning. Furthermore, NMDAR phenotype changes in parallel with developmental and seasonal periods for vocal plasticity. We also review recent studies in the avian song system that challenge the popular notion that sensitive periods for learning reflect developmental changes in the NMDAR that alter thresholds for synaptic plasticity.

KEYWORDS: NMDA receptor; development; sensitive period; learning; birdsong

Many behaviors are shaped by specific sensory and/or hormonal events that occur during "sensitive" periods in life. For example, avian song learning, human language acquisition, the development of normal sensory function, sexual differentiation, and imprinting all exhibit periods when experience has a particularly profound effect on brain organization and behavior. It is generally assumed that sensitive periods arise because experience affects the outcome of specific cellular and synaptic changes that occur uniquely, or are at least exaggerated, during neural development. In some cases, those aspects of neural development that may constrain sensitive periods are obvious. For instance, the onset of a sensitive period depends minimally upon the establishment of circuitry competent to convey the learning stimulus, the presence of modulatory pathways or molecules that enable learning mechanisms, and the expression of the intracellular machinery that produces lasting neural and behavioral

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change. Also, in at least some cases, closure of a sensitive period is relatively straightforward. For example, during sexual differentiation, the sensitive period for some aspects of hormone-induced masculinization is limited by the time course of naturally occurring cell death, because in some regions hormones rescue cells that would otherwise die.[1,2] However, with regard to sensory development and early learning, the complexity of synaptic and intracellular mechanisms that promote change in neural and behavioral organization create a considerable challenge for identifying neural events that regulate the onset and termination of sensitive periods. Significant progress in understanding the neurobiology of sensitive periods has come from studies of the developing visual system. This literature has provided strong evidence that specific forms of sensory input early in life permanently shape neural circuits by driving an activity-dependent process of synaptic strengthening, sprouting, weakening, and elimination.[3–5] Such experience-dependent synaptic rearrangement also is evident during imprinting[6–8] and likely exploits forms of synaptic plasticity that have been implicated in adult learning. Thus, research directed toward understanding sensitive periods has focused on characterizing age-related changes in the thresholds for inducing synaptic plasticity. However, changes in synaptic strength entail multiple interacting biochemical cascades and are subject to a myriad of modulatory influences. Thus, while this review focuses on a narrow set of changes in the molecular machinery that could directly have an impact on synaptic transmission and plasticity, it is important to recognize that multiple aspects of neural development affect thresholds for plasticity, and that the consequence of one developmental change will interact with the state of other relevant influences. In addition, synaptic strengthening and weakening is apt to have a more enduring affect on neural circuitry at times of rapid synaptogenesis and synapse elimination. Undoubtedly, sensitive periods are defined by the interaction of many events, and it is certainly possible (indeed likely) that no single aspect of development will prove sufficient to explain the timing of a given sensitive period.

RESEARCH STRATEGY AND TACTICS

Notwithstanding these complexities, a realistic hope is that certain developmental events produce powerful enough effects on synaptic plasticity so as to be able to measure their contribution to regulation of a sensitive period. From among the mix of events that overlap with any particular sensitive period, one can ask: which are necessary, and are any sufficient, to dampen plasticity? Generally, these questions are approached by first identifying the cellular mechanisms mediating a specific instance of developmental plasticity. There is an underlying prescription that the proposed cellular/molecular processes should be localizable and measurable in relevant brain regions during periods of learning. Moreover, if the candidate process is indeed necessary for the learning process, interfering with its function should impair behavioral indices of learning. Genetic or pharmacological manipulations are generally employed to test function, but their interpretation can be difficult because frequently they lack adequate specificity and may activate compensatory processes that can obscure the normal physiological role of a particular process. Despite these limitations, such studies add critical information because correlative studies alone are not sufficient to determine what processes are indeed necessary for learning. Only after a

specific cellular, synaptic, or molecular change has been directly implicated in learning can one assess whether qualitative or quantitative changes in its expression modulate plasticity. And, of course, regulation of plasticity can occur through changes in any aspect of the biological cascade that transduces sensory experience into lasting neural and behavioral change: (1) upstream circuitry that provides information to the learning mechanism (e.g., balance of inhibitory and excitatory inputs or neuromodulatory pathways), (2) the molecular machinery that provokes synaptic plasticity (e.g., receptors, intracellular signaling molecules, trophic factors), or (3) downstream events involved in expression of those plasticity cascades (e.g. , specific patterns of gene expression, synapse formation or elimination). Thus, several criteria are needed to evaluate whether any particular developmental change results in a sensitive period for learning. We need to ask (1) whether the developmental regulation coincides with the timing of the sensitive period, (2) whether variables that regulate the timing of the sensitive period regulate, in parallel, the expression of the candidate mechanism, and (3) whether manipulations that alter the time course of the candidate mechanism predictably alter the temporal profile of the sensitive period.

Over the past 10–15 years, the *N*-methyl-D-aspartate subtype of glutamate receptor (NMDAR) has come under close scrutiny as one potential regulator of sensitive periods. Attention has focused on NMDARs both because they are critical for many forms of learning and synaptic plasticity and because they are developmentally regulated in ways that seem to alter thresholds for plasticity. Here, we review evidence that avian vocal plasticity involves NMDAR-mediated forms of synaptic plasticity, with special emphasis on experiments that have investigated whether changes in NMDAR phenotype constrain the timing of this learning.

THE *N*-METHYL-D-ASPARTATE RECEPTOR AND SYNAPTIC PLASTICITY

There is general agreement that experience is stored in the brain as a consequence of an activity-dependent process of synaptic strengthening and weakening, governed in turn by the relationships between presynaptic activity and postsynaptic firing. Synaptic strengthening (manifest as long-term potentiation, LTP) and stabilization occurs preferentially at sites where presynaptic activity consistently contributes to firing the postsynaptic cell ("Hebbian" modification). In contrast, synaptic weakening (manifest as long-term depression, LTD) occurs when such correlations in pre- and postsynaptic activity are sparse or absent. In both young and adult animals, NMDARs participate in many such forms of bidirectional synaptic plasticity[9–11] and learning.[12–16] NMDARs are uniquely suited to detect such correlations in pre- and postsynaptic activity patterns because their channel is fully activated only when presynaptic release of glutamate coincides with significant postsynaptic depolarization.

The chemical and electrical gating that characterizes NMDARs is illustrated schematically in FIGURE 1. At resting potential, presynaptic release of glutamate opens the channel but does not result in significant current flow because the NMDAR channel is subject to a voltage-gated Mg^{2+} block. However, when the postsynaptic membrane is sufficiently depolarized, the Mg^{2+} dissociates from its binding site within the channel, and if glutamate is still bound when this occurs then the channel is fully activated. NMDARs often are expressed at synapses also containing another

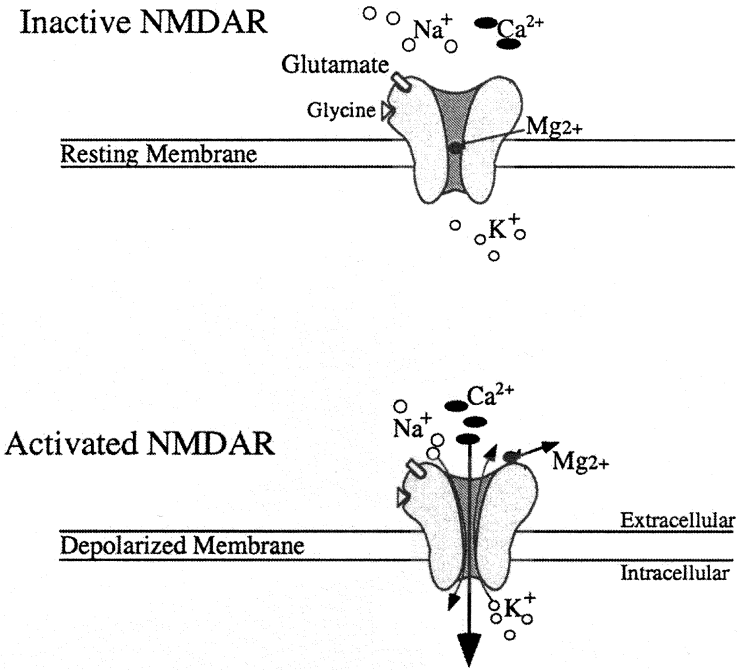

FIGURE 1. Voltage dependency of NMDAR function as compared to voltage-independent property of AMPAR function. Glutamate is the endogenous ligand for both receptor subtypes; NMDARs also contain several other binding sites (only glycine is shown here) that regulate channel function. NMDARs often coexist at synapses containing AMPARs, which mediate most of the initial depolarization from resting potential. As membrane depolarization increases, NMDARs become activated and can therefore contribute to late phases of the EPSP. See text for further details.

glutamate receptor subtype, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), which mediate most of the postsynaptic depolarization when membranes are close to resting potential. An additional critical feature of the NMDAR channel is that it is permeable to Ca^{2+} (as well as to Na^{+} and K^{+}).

Changes in intracellular Ca^{2+} regulate a variety of biochemical cascades important for long-term changes in synaptic function, and Ca^{2+} entry through NMDARs appears to be a critical trigger for many forms of synaptic plasticity.[17–20] Importantly, most synapses that express LTP also express LTD, and Ca^{2+} influx via NMDARs has been linked to both of these forms of synaptic plasticity. Among the multiple factors that can have an impact on the magnitude and sign of synaptic change, the amplitude and duration of the Ca^{2+} signal are important. Because of differential effects on protein kinase and phosphatase cascades that generally act in opposition to one another, a relatively large sustained rise in $[Ca^{2+}]$ increases the probability of synap-

tic strengthening while a more modest rise may instead facilitate synaptic weakening.[21–23] Importantly, the crossover point between LTD and LTP (the LTD/LTP modification threshold) can itself change as a function of the recent history of activity at the synapse. As average activity increases, the threshold for inducing LTP is raised while the probability of inducing LTD is increased, resulting in a form of "metaplasticity."[24]

In the developing nervous system, the various forms of NMDAR-mediated plasticity may help shape key patterns of neural organization (such as topographic mapping), through the competitive, activity-driven rearrangement of synapses.[25,26] Given their importance to early learning and plasticity, attempts to account for the timing of "sensitive periods" have focused on the regulation of molecules that could impact thresholds for NMDAR-mediated LTP or LTD. First among such molecules is the NMDAR itself: studies in a variety of neural systems have revealed robust developmental regulation of NMDAR structure and function that generally coincides with sensitive periods for neural and behavioral plasticity.[27–31] Moreover, it is well established that changes in the expression of specific NMDAR subunits alter NMDAR current duration[32,33] and modify interactions with intracellular proteins,[34–36] either of which could modulate selective synapse stabilization.

AVIAN SONG LEARNING—A MODEL OF DEVELOPMENTALLY REGULATED LEARNING

Developmental studies of the visual system have yielded tremendous insights into the neurobiology of critical periods for sensory/perceptual development, but it is not yet clear whether similar formulations apply to more complex instances of developmentally regulated learning. Neuroethological studies have described several instances of learning that exhibit well-defined sensitive periods, and avian song learning in particular has several features that make it a powerful paradigm for investigating cellular substrates of learning and their developmental/temporal regulation. First, birdsong is mediated by a well-characterized neural circuit, discrete portions of which are implicated specifically in vocal learning and behavior. Second, many birds exhibit strong stimulus biases in song learning (see Adret, this volume). Third, while many songbirds can only imitate songs heard during a distinct developmental period, there is tremendous species diversity both in the timing, and even the existence of, sensitive periods for avian vocal learning. And, even in "age-limited" vocal learners, the timing of the sensitive period can be extended by early isolation from conspecific song,[37–39] allowing one to dissociate chronological age from the system's ability to support vocal learning. These natural stimuli and temporal constraints, which are rarely available in more traditional paradigms of learning and memory, provide powerful tools for discriminating between cellular events specifically related to learning and those involved in more general aspects of sensory processing, motor activity, or maturation. Current evidence is consistent with the hypothesis that at least some aspects of avian song learning engage experience-dependent forms of NMDAR-mediated synaptic change that have been associated with other instances of developmental plasticity and learning.

CELLULAR AND BIOCHEMICAL SUBSTRATES

Brain regions mediating vocal learning in songbirds form two intimately related circuits (see Fig. 2 in Reiner *et al*., this volume); one pathway is necessary for song production, and the other is more specifically involved in song development and plasticity. Both of these circuits likely are involved in auditory-based vocal learning because they both contain auditory-sensitive neurons whose response selectivity reflects learned features of song, and both exhibit motor activity related to song production.[40,41] The "motor pathway" consists of several hierarchically organized neural regions (Uva-Nif-HVC-RA-motoneurons) that are necessary for song production (see Wild, this volume); damaging or stimulating this circuitry immediately disrupts song behavior in adult birds.[41,42] Importantly, this motor pathway provides a major input to, and receives the major output from, the other song-related circuit, the anterior forebrain pathway (AFP). Regions of the AFP form a basal ganglia-thalamocortical loop that has been implicated directly in song learning (see chapters by Bottjer, Brainard, and Perkel, this volume). This loop indirectly connects HVC to RA, and it consists of Area X, DLM, and lMAN. Area X, which is an avian homologue of the mammalian basal ganglia, also receives a large dopaminergic projection from the ventral tegmental area.[43,44]

Several observations indicate a role for AFP structures in song memorization (sensory acquisition) and/or sensorimotor learning (vocal practice). In zebra finches (*Taeniopygia guttata*), lesions of Area X,[45,46] DLM,[47] or the lMAN[46,48,49] do not disrupt the production of stable song behavior in adults, but permanently impair the development of song in young males. In fact, even extended learning, as occurs in isolation-reared birds, depends upon an intact lMAN.[50] Recently, a model of AFP function has been postulated in which the AFP circuit provides a permissive or instructive "error signal" that promotes behavioral change when a mismatch is detected between expected auditory feedback and the stored song template.[51] This model is consistent with the fact that both motor and auditory information is available to the AFP, and that lesions of the lMAN prevent the vocal change that occurs in adult zebra finches when auditory feedback is eliminated or distorted.[52,53]

A possible contribution of NMDARs to avian song behavior was first suggested by descriptions of regional NMDAR expression patterns and physiological characteristics within the song system of zebra finches. NMDARs were first identified at the lMAN–RA synapse, where they mediate the majority of the synaptic current in both young and adult males.[54] Shortly thereafter, receptor binding studies revealed the presence of NMDARs in other song-related brain regions, including expression in HVc, Area X, and lMAN.[55] Notably, developmental regulation of overall binding (using the noncompetitive NMDAR antagonist, MK-801) was detected only within the lMAN. Males still within the sensitive period for song learning exhibited elevated MK-801 binding in this region, and normal song development was associated with a gradual decrease in the number of lMAN NMDARs and an increase in their affinity for MK-801.[56]

Evidence of age-related changes in NMDAR expression within the lMAN was particularly interesting both because this region is critical for normal song development and because synapses here dramatically reorganize in response to experience during the sensitive period for song acquisition. That is, glutamatergic inputs arising from DLM expand greatly in young zebra finches between 20 and 35 days posthatch

and then steeply reduce their terminal fields.[57,58] These anatomical modifications in zebra finches overlap with both sensory acquisition (occurring between ~25–60 days posthatch), and sensorimotor learning (occurring between ~35–120 days posthatch). As the DLM terminals are pruned within lMAN, there is a corresponding reduction in the density of dendritic spines (postsynaptic specializations) on lMAN neurons.[59] Furthermore, this pruning of dendritic spines is delayed in young birds isolated from conspecific song.[60] Given the involvement of NMDARs in many forms of learning, as well as the relationship between memory formation and the loss, addition, and modification of spines,[8,61,62] it was a logical step to test directly whether NMDAR activation is necessary for normal song development.

NMDARs AND SONG ACQUISITION

We used a pharmacological approach to test whether NMDARs are critical for sensory acquisition. First, we found that systemic blockade of NMDARs impairs song learning when it overlaps with restricted periods of song tutoring, but not when it occurs on nontutoring days.[63] Birds injected with the NMDAR antagonist just prior to tutoring developed songs that resembled those of birds never exposed to a song model. Subsequently, we found that song learning is compromised when NMDARs are blocked specifically within the AFP during periods of song exposure.[12] As shown in FIGURE 2, birds receiving infusions of the NMDAR antagonist AP5 directly into lMAN immediately prior to tutoring sessions ultimately reproduced significantly less of the tutor's song than did various different control groups, including birds that received identical infusions of AP5 on nontutoring days.

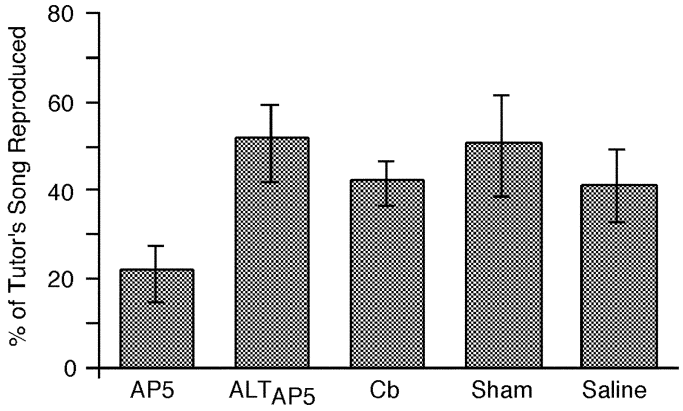

FIGURE 2. Percentage of tutor's song reproduced in five groups of 90-day-old zebra finches. Two groups received bilateral injections of the NMDA receptor antagonist AP5 into the lMAN either 10 min prior to tutoring (AP5) or on nontutoring days (ALT_{AP5}). Other birds received injections of either AP5 into the cerebellum (Cb) or saline into the lMAN (Saline) 10 min prior to tutoring, or were left uninjected (Sham). Tutoring sessions (90 min) occurred every other day from posthatch day 32–52. (From Basham *et al.*[12])

These data suggest that NMDAR activation in the AFP is necessary for normal song learning, perhaps because they mediate processes involved directly in encoding and/or storing the song template. Other interpretations are possible, however. The behavioral deficits could reflect impairments of vocal practice (*sensorimotor learning)* as opposed to acquisition, because both of these phases of song learning overlapped with our AP5 treatments. However, because birds experiencing NMDAR blockade on nontutoring days produced normal songs, this interpretation requires the added presumption that normal vocal learning requires periods when vocal practice actually coincides with tutor exposure. While this opportunity does normally exist for zebra finches, a high level of tutor imitation can be achieved in this species even when exposure to conspecific song terminates before, or shortly after, the onset of vocal practice.[64,65] Moreover, in other songbird species, sensory acquisition and the onset of sensorimotor learning can be separated by many weeks or months.[66] Thus, the most parsimonious interpretation is that NMDAR activation within the AFP is critical to template encoding, in addition to any role these regions (and NMDARs) also may play in sensorimotor learning.

Another consideration is that NMDAR blockade within the AFP may disrupt acquisition by interfering with fast excitatory transmission, rather than NMDAR-mediated synaptic plasticity *per se*.[67] While this possibility is difficult to rule out, *in vitro* recordings from lMAN neurons suggest that significant synaptic transmission persists even in the presence of NMDAR antagonists. That is, AP5 produces only a small reduction (~13%) in the peak amplitude of EPSPs evoked by DLM stimulation, but a somewhat larger peak amplitude reduction (~25%) of EPSPs evoked by stimulation of lMAN recurrent axon collaterals.[68–70] Importantly, several additional observations also support the idea that NMDARs within the AFP mediate synaptic plasticity during song learning. First, NMDAR-mediated LTP has been documented in both lMAN and Area X.[71,72] In lMAN, NMDAR-mediated LTP induced at the recurrent collaterals of lMAN neurons is accompanied by synaptic weakening at the DLM synapses,[72] and both of these forms of plasticity can be induced only during the sensitive period for song memorization. Because synaptic weakening at the DLM–lMAN synapse only occurs when the DLM input is inactive during postsynaptic activation, the authors suggest that the LTD may ultimately lead to the pruning of ineffective DLM afferents. As noted before, song learning is accompanied by both pruning of DLM afferents to lMAN[57,58] and elimination of dendritic spines on lMAN neurons that is modulated by exposure to conspecific song.[59,60]

Additional evidence that song exposure during the sensitive period may involve NMDAR-mediated plasticity within the AFP comes from recent biochemical studies we have initiated to investigate whether calcium-calmodulin–dependent protein kinase II (CaMKII) is critically involved in song learning. The activation of this protein is an event that is downstream of NMDAR activation: CaMKII is abundant in the postsynaptic compartment, it is activated (phosphorylated) by synaptically driven Ca^{2+} elevations, and CaMKII activity is critical for the induction of NMDAR-dependent LTP, as well as certain forms of learning and developmentally regulated synaptic plasticity.[73] In young zebra finches previously denied access to conspecific song, two hours of song exposure increases levels of phosphorylated CaMKII (pCaMKII) within Area X of the AFP.[74] Moreover, in juveniles exposed to song only until posthatch day 30, two hours of reexposure to familiar song promotes

a robust elevation in pCaMKII within this region (unpublished observations). As noted earlier, Area X is essential for normal song development, and is a part of the avian basal ganglia that receives a major input from both HVC and lMAN. While this region exhibits both auditory and motor-related activity, the pCaMKII signal detected is not a reflection of vocal practice since it occurs even in the absence of juvenile song production. Remarkably, in birds exposed to song until day 30, exposure to an *unfamiliar* song does not elicit an elevation in pCaMKII. While the reasons for the differential effects of familiar versis unfamiliar song are not yet clear, one possibility is that it relates to modifications in synaptic strength resulting from earlier exposure to a particular song. Song exposure only until posthatch day 30 is sufficient to selectively tune neurons within the AFP to song patterns previously heard,[75] and this tuning presumably reflects the selective strengthening of pathways activated by that prior song exposure. Thus, exposure to familiar song should reactivate potentiated pathways and thus be optimal for activating CaMKII, whereas unfamiliar song would activate nonpotentiated or weakened pathways and thereby may produce insufficient postsynaptic activation to elicit a detectable pCaMKII signal. While we have not yet tested directly whether NMDAR activation is necessary for this pCaMKII signaling, or whether the pCaMKII signal is essential for normal song learning, these are important future directions for research.

CHANGES IN THE COMPOSITION AND FUNCTION OF NMDA RECEPTORS DURING SONG LEARNING—A SUBSTRATE FOR THE SENSITIVE PERIOD?

Developmental studies in a variety of vertebrate neural systems support the view that sensitive periods for plasticity are characterized by heightened susceptibility to Hebbian-like forms of synaptic change.[31,76] Likewise, constraints on the timing of song learning suggest that neural function is modified by development or season so as to affect the ability of auditory experience to shape song circuits. This suggestion is consistent with the observation that the ability to induce LTP and LTD at synapses within the lMAN normally declines in juvenile zebra finches as development proceeds.[72] In fact, the same stimulus parameters that induce LTP in young birds elicit LTD in adults, suggesting that the LTD/LTP modification threshold has shifted in favor of LTD. One of the many factors (e.g., see refs. 77 and 78) that can regulate thresholds for synaptic change is experience-driven, developmental change in NMDAR composition and physiology.[29,30,79–82] In fact, over the past 10–15 years, change in NMDAR subunit expression has been proposed as a leading mechanism for curtailing plasticity as sensitive periods close.

Native NMDARs consist of the NR1 subunit, essential for channel activity, and one or more modulatory subunits (NR2A–E) that determine the biophysical properties of the receptor channel.[32,83,84] During the sensitive period for song learning, NR1 mRNA levels within lMAN decline,[85] thus downregulation of gene transcription likely drives the developmental decrease in MK-801 binding sites. Similarly, in mammalian visual cortex, several investigators have reported a decline in NR1 expression and MK-801 binding as NMDAR-mediated plasticity wanes.[79,86,87] While these quantitative changes in overall NMDAR expression correlate with reduced plasticity at the close of sensitive periods, they do not themselves curtail plastic-

ity. That is, they are insensitive to experiential manipulations that extend the sensitive period for either avian song learning or plasticity in the mammalian visual system.[56,85–88]

In contrast, some of the developmental changes in the NMDAR modulatory subunits and physiology are regulated by experience. NR2A and NR2B are the most prevalent modulatory subunits in vertebrate forebrain, and many regions express high levels of NR2B early in development and then gradually replace or augment these with NR2A subunits.[89,90] There are at least two distinct ways in which changes in NMDAR composition could affect thresholds for synaptic change. First, an increase in the NR2A:2B ratio decreases the decay time of NMDAR currents, thereby reducing the time course of NMDAR-mediated increases in postsynaptic Ca^{2+}.[32,33]

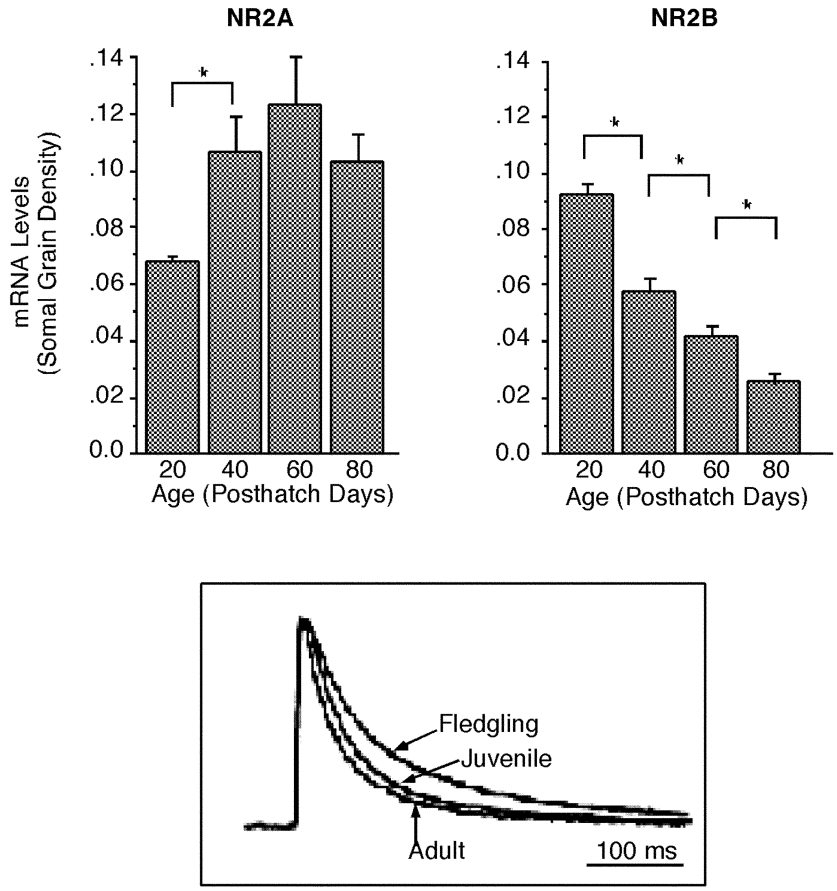

FIGURE 3. Developmental changes in the expression of NMDAR modulatory subunits NR2A and NR2B mRNA (*top*; modified from Singh *et al.*[85] and Heinrich *et al.*[95]) and physiology (*bottom*; modified from White *et al.*[93]) within the lMAN. As the NR2A:2B ratio increases, NMDAR currents within the lMAN become faster.

Second, NR2A and NR2B subunits associate differentially with various intracellular molecules that are involved with receptor localization and/or signaling cascades that regulate synaptic function.[35,91] Thus, changes in the expression of NMDAR modulatory subunits can affect the probability and outcomes of initiating specific signal transduction cascades involved in synaptic plasticity.

Within the avian song system, HVC, RA, Area X, and lMAN all exhibit a developmental increase in NR2A transcripts that accompanies a decrease in NR2B transcripts.[85,92] While the amplitude and timing of these changes vary somewhat across regions, the pattern in lMAN (FIG. 3, top) exemplifies the developmental regulation of these subunits within song regions. NR2A transcripts in lMAN increase relatively early in song development, while a reciprocal decline in NR2B mRNA tends to be more protracted. As noted earlier, an increase in the NR2A:2B ratio shortens NMDAR currents, and in both RA and lMAN (other regions have not been explored), the expected decrease in NMDAR current durations have been confirmed (FIG. 3, bottom).[68,72,93,94]

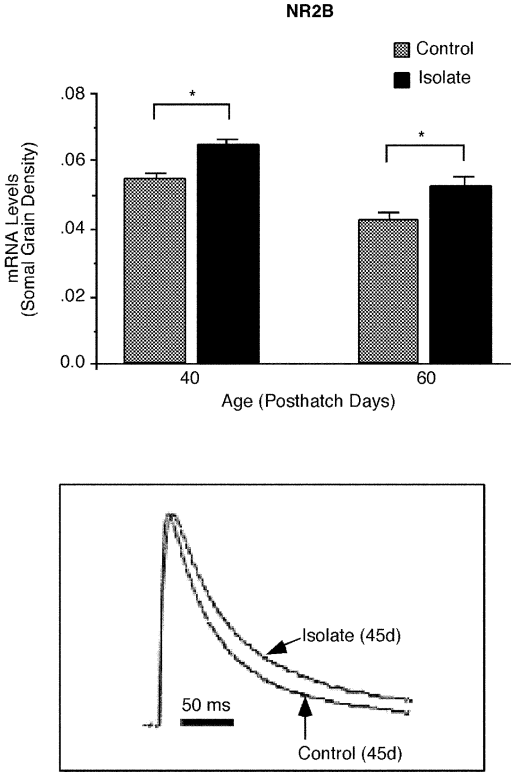

FIGURE 4. Early isolation delays the developmental decline in NR2B mRNA expression within lMAN (*top;* modified from Singh *et al.*[85]). Correspondingly, this manipulation also delays the developmental decline in the duration of NMDAR currents within the lMAN (*bottom*; modified from Livingston *et al.*[99]).

The hypothesis that developmental changes in NMDAR subunit composition limit sensitive periods for experience-dependent plasticity grew primarily out of studies on visual system development. Here, not only does the sensitive period for monocular deprivation coincide with changes in the NR2A:2B ratio and NMDAR current durations, but manipulations that extend this sensitive period also delay the developmental increase in NR2A subunit expression and the shortening of NMDAR currents.[29,96–98] Several years ago, it also was shown that within the lMAN of the avian song system, maturational changes in NMDAR expression and physiology are affected by manipulations that alter the timing of the sensitive period for vocal imitation. That is, early isolation from song delays the decrease in NR2B mRNA expression[85] and delays the developmental decrease in NMDAR current durations within this region.[99] These observations are illustrated in FIGURE 4: at both 40 days and 60 days posthatch, NR2B transcripts in lMAN are elevated in isolates relative to controls, and at 45 days isolates have slower NMDAR currents in lMAN than do age-matched controls. Interestingly, these effects of early experience are quite targeted—isolation does not alter NR2B mRNA expression in other song regions, nor does it affect NR2A mRNA expression in any region examined (lMAN, Area X, HVC, or RA).

Developmental changes in the NR2A:NR2B ratio and NMDAR physiology in lMAN also are regulated by hormones. They are accelerated by early testosterone treatment,[85,95,100] which impairs song development in zebra finches[101] and can both accelerate and disrupt learning in other age-regulated learners.[102] In fact, changes in NMDAR composition within the lMAN even map onto seasonally recurring periods of song plasticity in adult canaries. NR2B mRNA levels within the lMAN are higher during short-day photoperiod conditions when testosterone levels are low and song is being actively remodeled, than when measured under long-day conditions when testosterone levels are elevated and song structure is stable.[103] In contrast, NR2A mRNA expression in lMAN is similar under short- and long-day photoperiods.

While these intriguing brain/behavior relationships fueled the hypothesis that declining NR2B expression may contribute significantly to sensitive period closure, recent studies directly testing this idea have failed to confirm the hypothesis. As previously noted, early isolation from conspecific song delays developmental changes in the expression of NR2B mRNA within the lMAN, while also extending the sensitive period for learning. However, recently we found that either limited exposure (up until day 30) to a tutor's song, or brief exposure (day 24–30) to testosterone, restores in isolates a normal time course to these developmental changes in NMDAR gene expression within lMAN *without compromising extended learning*.[104] As shown in FIGURE 5, NR2B mRNA levels at 65 days were at, or even somewhat below, normal levels in these experimental groups. And yet both imitated the song of a tutor first encountered at 65 days while control birds did not. Although it is possible that the sensitive period remains open in these experimental birds due to post-transcriptional changes that enable high levels of synaptic NR2B protein despite depressed mRNA levels,[105] it should be noted that closure of the sensitive period for song acquisition also has been dissociated from developmental changes in lMAN NMDAR physiology. That is, T-treated isolate zebra finches can acquire new song material even well after NMDAR currents within lMAN have matured to their adult duration.[99]

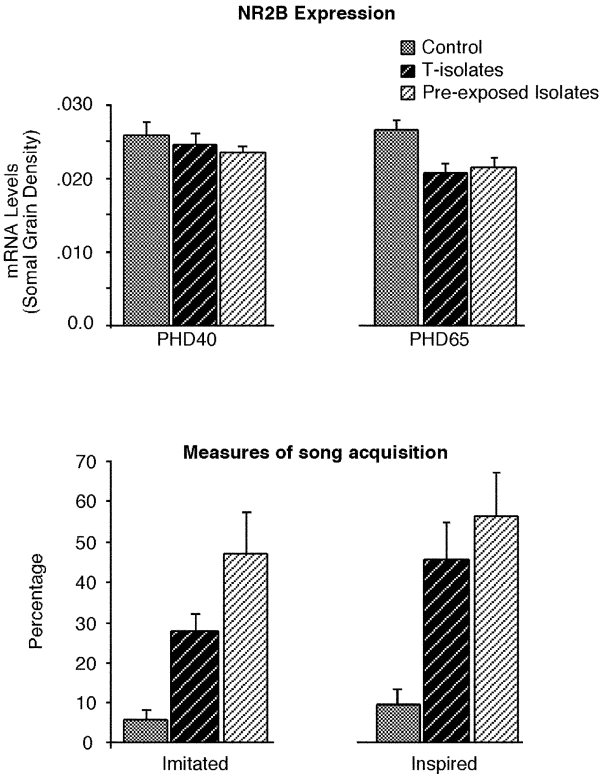

FIGURE 5. Isolate birds that are either treated with testosterone from days 24–30 (T-treated isolates), or exposed to song only until 30 days posthatch (preexposed isolates) have NR2B transcript levels within lMAN that are at, or below, the normal range of age-matched controls (*upper panel*). Yet, these two groups of isolates remain capable of imitating a significant amount of song first heard after day 65, while control birds are not (*lower panel*). "Imitated" reflects the amount of Tutor song reproduced by the pupil; "Inspired" reflects the amount of pupil song scored as learned (modified from Heinrich *et al.*[104]).

Although it is now clear that developmental regulation of NMDARs cannot account for the close of the sensitive period for vocal imitation, it would be premature to conclude that such regulation normally does not impact vocal learning. That is, our measures of how much song was imitated after a protracted period of tutoring are relatively crude, and it is entirely possible that small, but nevertheless important changes in learning propensity have gone undetected. Also, our studies do not address whether NMDAR maturation is a *necessary* component of sensitive period regulation. Investigations of developmental plasticity in the developing somatosensory and visual cortex clarify this distinction. By employing genetic knockouts, these studies have shown that deletion of the NR2A subunit does not impact the normal time-course of the sensitive period for monocular deprivation[106] or for synaptic and

anatomical plasticity in barrel cortex.[107] Thus, the maturational increase in NR2A and the shortening of NMDAR currents are neither necessary nor sufficient for the opening and closing of these sensitive periods. However, the NR2A deletion does weaken overall sensitivity to monocular deprivation, and also impacts the timing of the sensitive period for development of orientation selectivity in visual cortex.[106] These observations bring into sharp focus the diversity of sensitive periods and emphasize the need to independently establish for any particular sensitive period the unique set of determinants that dictate responsiveness to specific patterns of input.

CONCLUSIONS AND FUTURE DIRECTIONS

While the last decade has seen most emphasis placed on the hypothesis that higher NR2A:NR2B ratios and faster NMDAR currents should reduce plasticity and curtail sensitive periods,[29,108–111] other evidence favors the hypothesis that these early changes in NMDAR phenotype actually may contribute to *opening* the sensitive period. In both ferret and rat visual systems, the NR2A:NR2B ratio increases and receptor currents quicken at the *onset* of the sensitive period, just as visual experience begins patterning synaptic organization.[112,113] Furthermore, while exposing dark-reared animals to light rapidly increases the NR2A:2B ratio and shortens NMDAR currents,[96,97] these changes clearly do not close the sensitive period, because dark-reared animals are sensitive to the effects of monocular deprivation for many days after first exposure to light. Finally, postponing NMDAR maturation by dark-rearing delays not only closure but also the *onset* of the sensitive period for visual cortical plasticity.[114]

In the song system as well, the most precipitous change in the NR2A:NR2B ratio and NMDA current duration in lMAN occurs between posthatch days 20–40, near the onset of the sensitive period for sensory acquisition and vocal motor practice. Because an increase in neural activity can facilitate maturation of NMDAR subunit expression,[96–98,115] the increased circuit activation associated with either phase of song learning could drive changes in NMDAR composition and physiology which, in turn, may raise the LTD/LTP modification threshold. This could help ensure that only precisely correlated activity patterns trigger NMDAR-mediated synaptic stabilization. Thus, initial exposure to song may "prepare" neural circuitry for encoding the rich temporal and acoustic structure of song, by fostering a period of rapid, experience-driven synapse elimination that is the hallmark of other sensitive periods. This view predicts that manipulations of the early auditory and endocrine environment that delay changes in NMDAR expression and function should likewise delay the *onset*, as well as the closure of the sensitive period for learning.

As noted earlier, the myriad of factors that can affect neural plasticity make it improbable that any single maturational change is responsible for limiting the timing of avian vocal learning. Yet, while the biology accounting for sensitive periods remains elusive, the established role of NMDAR activation in song learning, coupled with the insight provided by work on a variety of other instances of developmental plasticity, suggests two broad avenues for future research. The first of these should continue to investigate age-regulated processes that could modify thresholds for those forms of activity-dependent synaptic change most commonly thought to underlie learning and experience-dependent plasticity**.** For example, developmental

changes in inhibitory circuitry could regulate sensitive periods by enabling and then constraining LTP/LTD-mediated synaptic change. Data from mammalian visual cortex suggest that a certain threshold level of inhibition may be permissive for sensitive period plasticity, but subsequent increases in inhibitory (relative to excitatory) transmission can reduce plasticity.[116] Developmental changes in parameters of inhibition within the song system have not been well characterized with respect to the timing of the sensitive period. Calecholaminergic and cholinergic systems also can modulate plasticity,[78,117] and within song nuclei there are striking developmental changes in the expression of these neurotransmitter systems that could modulate synaptic plasticity within the AFP or elsewhere.[118–121] And further work on NMDAR-linked biochemical cascades activated during sensory acquisition may reveal maturational changes in synaptic or nuclear signaling enzymes that could limit learning. For example, CaMKII,[122] extracellular signal-related kinase,[123] and protein kinase A[124] activation have all been associated with plasticity in the visual cortex of developing mammals. Alternatively, the regulation of molecules associated with forgetting or suppressing learning and memory,[125,126] could interfere with forming new representation of song. More specifically, their action could curtail the "rewritability" of song circuitry tuned to a particular song, and thus contribute to the end of the sensitive period for acquisition.

A second line of research should probe how cellular processes unique to (or at least exaggerated in) the developing brain might alter the impact of synaptic plasticity on circuit organization. For example, the normal overlap between sensory acquisition and synaptic pruning in lMAN may facilitate information storage (but see ref. 127) as suggested by recent studies of visual imprinting.[8,128] Another likely substrate for synaptic plasticity is the potential for novel synapse formation, or the formation, modification, and elimination of dendritic spines that do not necessarily result in a net gain or loss in synapse number. The rate of dendritic spine turnover in visual cortex is significantly higher throughout the sensitive periods for plasticity than it is in adulthood.[129] Yet, when portions of the extracellular matrix known to restrict dendritic spine motility are artificially decomposed, sensitivity to monocular deprivation is reinstated in adults.[125] Also, in layer 2/3 of whisker barrel cortex where experience-dependent plasticity can persist into adulthood, a physiological response to whisker manipulation is correlated with an increase in the rate of dendritic spine turnover.[130] Finally, in virtually every developing neural systems where NMDAR-dependent LTP occurs (including the avian song system), many glutamatergic synapses are functionally "silent" (NMDAR only, therefore generating no response at resting potential), and the incidence of these silent synapses declines with age.[131,132] Moreover, a growing body of evidence suggests that NMDAR activation can convert these silent synapses into functional excitatory connections by promoting the insertion of AMPA receptors into postsynaptic sites (see ref. 132 for review). The ability for activity related to the processing of song stimuli to recruit subthreshold synapses into functional ones could be essential for auditory experience to shape song circuitry.

In exploring these hypotheses it will continue to be important to move beyond purely correlative studies. Identifying relevant maturational events whose time course can be manipulated in parallel with the sensitive period will, of course, remain a valuable approach. But developmental investigations of NMDAR composition and physiology emphasize the need for manipulations that alter the timing of

song learning while minimizing gross effects on system maturation. The early auditory, visual, social, and endocrine experiences so important to vocal learning undoubtedly also have an impact upon a variety of developmental processes not specifically involved in regulating early learning. When these stimuli are withheld completely, a host of maturational consequences can be expected, only some of which may contribute to sensitive period regulation. One useful approach exemplified by our recent studies is to employ gentler manipulations of early input so as to alter the timing of learning without necessarily altering all developmental changes that depend on that input. This will at least narrow the field of processes identified as candidate mechanisms for closing the sensitive period. Finally, as this approach uncovers developmentally regulated molecular changes that *reliably* parallel the sensitive period, emerging techniques for molecular and/or genetic manipulation can target these changes directly and individually in order to assess their contribution to sensitive period regulation.

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