

## **LECTURE 3:**

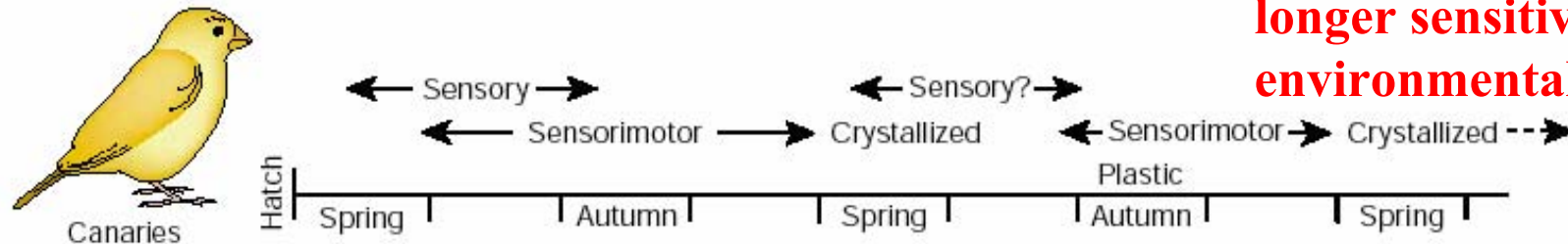
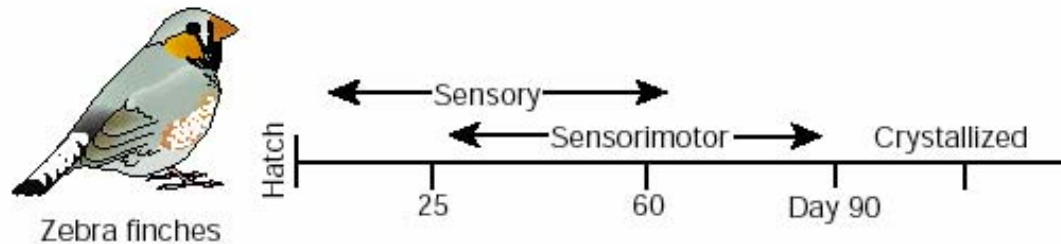
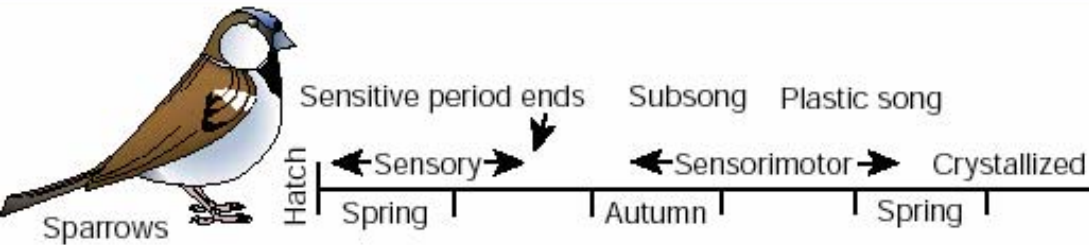
# **Cellular and Molecular Mechanisms of Song Learning In Birds**

## Plasticity Can be Regulated By Gonadal Steroids:

1. Several nuclei in passerine birds that are involved in song production (HVC and RA) are sexually dimorphic being much larger in males. Males sing conspecific songs to attract appropriate mates.
2. Young female Zebra finches given early estradiol treatment (known to masculinize in many vertebrate species) develop large HVC and RA and sing almost as well as males (Gurney & Konishi, 1980; Simpson & Vicario, 1991).
3. In canaries that add new syllables to their song every spring, seasonal increases in testosterone levels are tightly correlated with seasonal song nuclei size increases.

# WHAT DOES BIRDSONG LEARNING INVOLVE ?

Different species of passerine birds learn the conspecific (or tutor song) on different time schedules but the stages are the same



**1. Sensory: Sensitive period for song learning. Acquisition phase forming an internal template of a tutor's song.**

**2. Sensorimotor: Matching the bird's own song (BOS) to the template. "Subsong" progresses to 'Plastic Song'.**

**3. Crystallized song: no longer sensitive to environmental cues.**

# Sonagrams (representing frequency across time) provide sensitive assays of the stages of song learning (ex. swamp sparrows)

“Tutor Song” training is begun in a laboratory. The 5 syllables are played to the bird only during his first summer. Sensorimotor learning begins the following spring when the bird is approximately 250 days old.

## Sensorimotor Learning

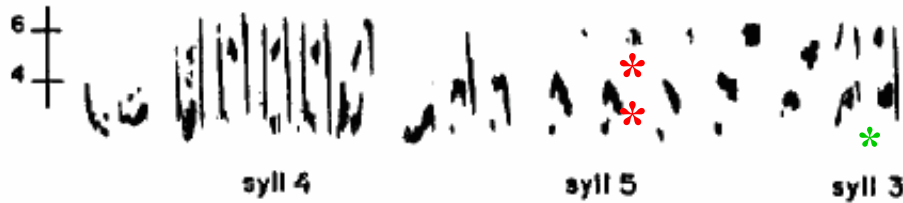


(b)

Subsong (Day 252)



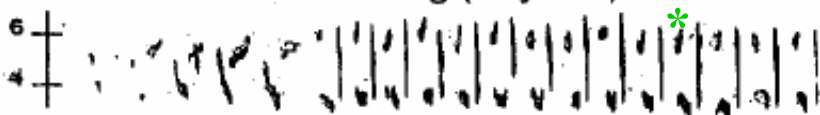
Plastic song (Day 260)



Plastic song (Day 279)



Plastic song (Day 279)



syll 1

syll 3

Plastic song (Day 287)



Crystallized song (Day 316)



# **1. Sensory: Sensitive period for song learning.**

## **A. Acquisition of sensory information occurs early in life but termination can also be influenced by environment.**

Zebrafinch raised in isolation even just visual and not acoustic isolation for up to 100 days “crystallize” an aberrant isolate song. However, these birds can still learn an isolate song reasonably well (Morrison & Nottebohm, 1991).

Song learning in Zebrafinch is disrupted when testosterone is given to < 40 day old birds during sensory to sensorimotor learning phase but not if it is given later in the sensorimotor phase (Korsia & Bottjer, 1991).

In sparrows, which have long delays between sensory/acquisition and sensorimotor learning, isolation can extend acquisition 6-8 months when photoperiods are adjusted to keep testosterone level low (Whaling CS et al. 1998).

## **B. Genetic factors can also play a role.**

Migratory and sedentary populations of sparrows differ in the length of their sensory/acquisition phase. Moreover, these differences persist in the laboratory when members of each population receive identical tutoring regimens (Nelson DA et al., 1995).

## 2. Sensorimotor Learning

**A. Early castration can prevent or delay song crystallization and the end of sensorimotor learning in several song bird species. However, early castration does not interfere with sensory/acquisition or the initiation of sensorimotor learning (Arnold AP,1975).**

**B. Attainment of a good match to template song does not determine the end of sensorimotor learning.**

Botulinum toxin injections into the song muscles reversibly paralyzes them and distorts song production. However, permanently abnormal song results from this procedure only when it is imposed very close to the time of song crystallization. Toxin injections early in sensorimotor learning or after song crystallization have no effect on matching of BOS to tutor song (Pyette & Suthers Soc Neurosci Abst. 1996).

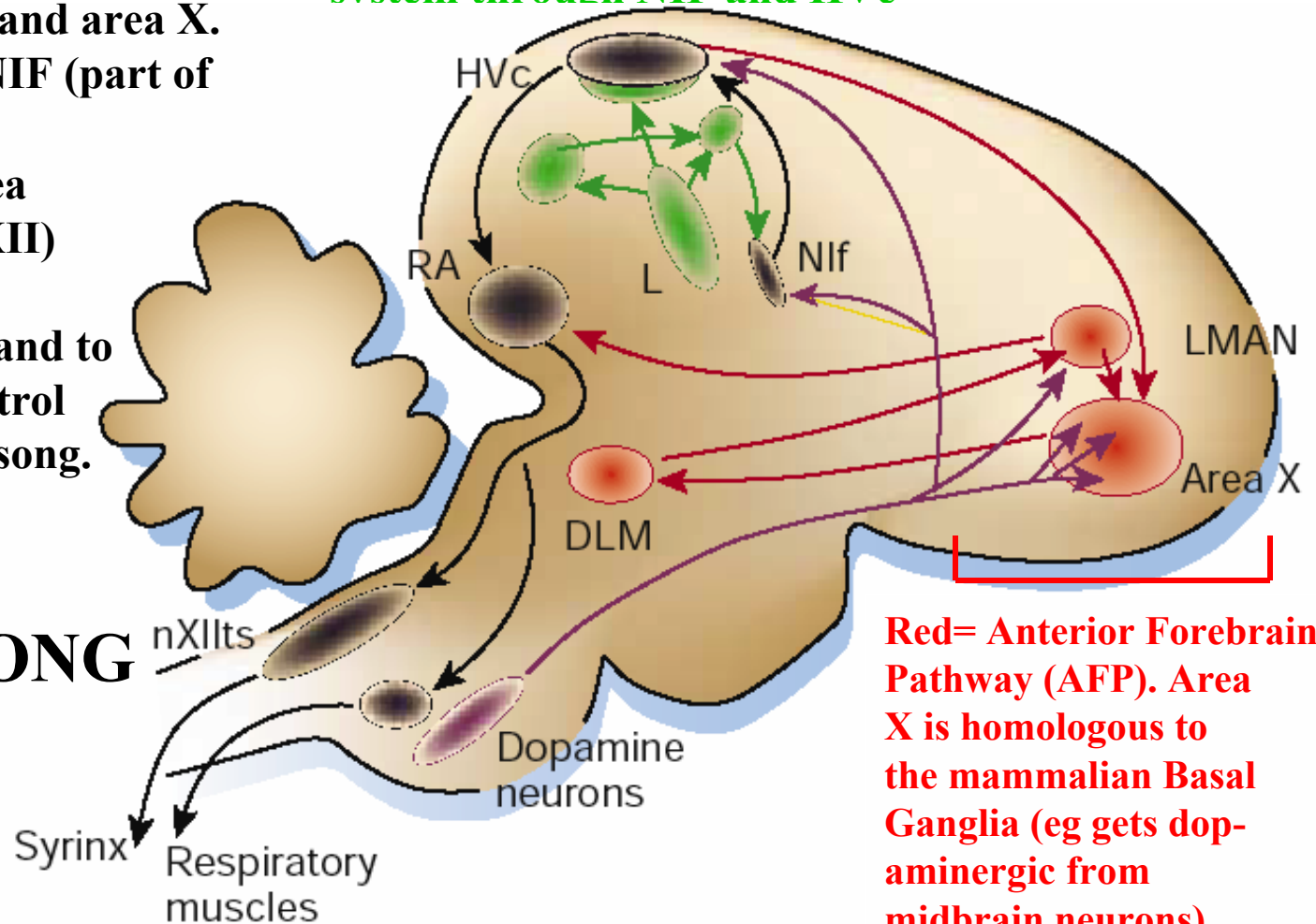
Isolate song also crystallizes

## 3. Crystallization

Crystallization represents a permanent commitment to a set of syllables. Some species annually re-crystallize their songs singing tutor syllables that they never incorporated into BOS. However, when crystallization is complete the bird goes back to using the same syllables it had used previously (Marler & Peters Auk 1982,99:446).

**Black = Motor pathway. Necessary for normal song throughout life**  
**HVC:** projects to RA and area X.  
**Receives input from NIF (part of motor pathway)**  
**RA projects to the area of the hypoglossal (nXII) that innervates the 6 muscles of the syrinx and to nuclei involved in control of respiration during song.**

**Green = auditory sensory areas. L is avian primary auditory area. Auditory input enters the song system through NIF and HVC**



## THE BIRD SONG SYSTEM

**Red= Anterior Forebrain Pathway (AFP). Area X is homologous to the mammalian Basal Ganglia (eg gets dopaminergic from midbrain neurons)**

**Area X also projects to dorsolateral thalamus (DLM) and to LMAN which is a “frontal cortex-like nucleus”. LMAN sends a projection to RA. This is a projection back to the motor pathway. Similar to the basal ganglion motor pathway projection in mammals.**  
**The projection between LMAN and RA is critical during motor learning.**

# **BACKGROUND FOR NMDA RECEPTORS AND BIRDSONG READINGS**



# How Do you Determine Where Synaptic Plasticity Is Implemented Pre- or Post-synaptic?

(Bekkers & Stevens, 1990, Nature 346: 724)

Three variables of quantal synaptic transmission from one pre-synaptic cell to one post-synaptic cell.

*Physiological event or state*

*variable*

1. Number of vesicle release sites present. Likely to be number of docked vesicles. Total number of available quanta.

**N**

2. Probability of release of 1 quanta of transmitter. Probability of release at 1 vesicle release site.

**p**

3. Size of the quantal response. Post-synaptic change as a result of the release of 1 vesicle. Generally estimated as the average size of mini's or, if quantal peaks are discernable, the smallest mini size.

**q**

**a**

5. Quantal content of any response

**m = aNp**

**blue = generally measured variables**

# Methods of Differentiating Pre-synaptic Site of Plasticity From Post-Synaptic Site of Plasticity at Synapses

**A. Do miniature (single quanta  $a$ ) synaptic events change in amplitude?**

**If yes where is the probable change?**

**Probably post-synaptic.**

**B. Paired pulse ratio (PPR) :** Stimulate input with pulses ~50 -100 msec apart. Measure EPSP1 and EPSP2.  $PPR = EPSP2/EPSP1$ . Or EPSC's 1 & 2  
This method is based on the observation that at relatively high frequencies of stimulation synapses with a high probability of vesicle release have a more depleted quantal content ( $m$ ) on the second stimulation than do synapses with a low probability of vesicle release.

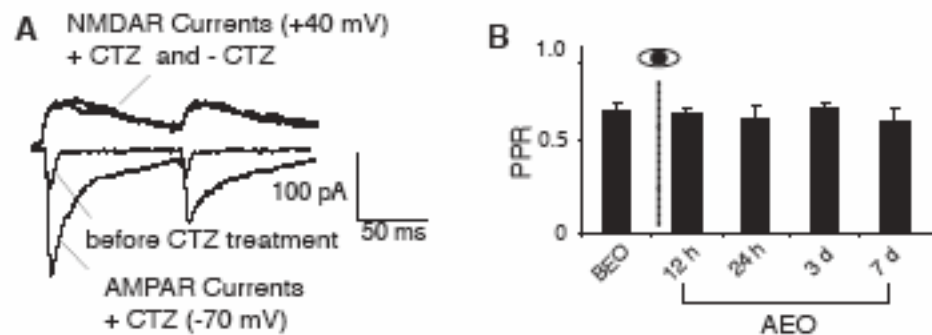


Fig. 10 Probability of transmitter release does not change at collicular synapses over the interval investigated.

(A) Pair-pulse stimulation with a 100 ms interpulse interval in the presence of AP5 and cytothizide (CTZ, 50 mM, to prevent AMPAR current desensitization) was used in this assay. The amplitudes of evoked AMPAR currents were measured. Recordings of AMPAR currents were made at -70 mV. Recordings of NMDAR currents were also made at +40 mV. The NMDAR currents did not change in the presence of CTZ, indicating that pre-synaptic effects of CTZ did not occur at these synapses (Chen et al., 2002). The average of 3-5 AMPAR responses to the paired-pulse stimuli were used to calculate the paired-pulse ratio (PPR) of given cell. The PPR was obtained by dividing the amplitude of the AMPAR current evoked by the second pulse by the amplitude of the AMPAR current evoked by the first pulse.

(B) Average PPRs from neurons recorded 1 day before BEO and over a range of time points AEO are shown. The PPR did not change over this interval. N = at least 7 neurons at each time point.

If you find that synaptic activation causes a switch from a PPR of 0.6 to a PPR of 1.0, what has happened to the probability of release as a result of the activity?

$$\text{PPR} = \text{EPC2}/\text{EPS1}$$

**Probability of release has been reduced.**

C. Coefficient of variation (CV) of response strength before and after the stimulation that induces plasticity.

Definition  $CV = \text{standard deviation} / \text{mean}$ ; standard deviation  $= \sqrt{\text{population variance}} = \sqrt{v}$   
 $CV = \sqrt{v/m}$ . Experimentally  $v$  is the variance of the sample of responses.

Remember quantal content,  $m = aNp$ .

High CV of post-synaptic response usually implies small  $m$ . Low CV usually implies a high  $m$ .

**If CV goes from small to large after the plasticity inducing stimulus has the quantal content of the response changed? Possibly**

**Is the change pre- or post-synaptic? Pre-synaptic only if  $a$  has not changed.**

Therefore look at mini EPSC amplitude frequency histogram.

If  $a$  has not changed (standard deviation for  $a$  usually does not change much) and CV after the plasticity inducing stimulation is lower than before then it is likely that  $Np$  (the probability of release) has increased

If  $a$  has not changed and CV after plasticity inducing stimuli is higher than before then it is likely that  $NP$  has decreased.

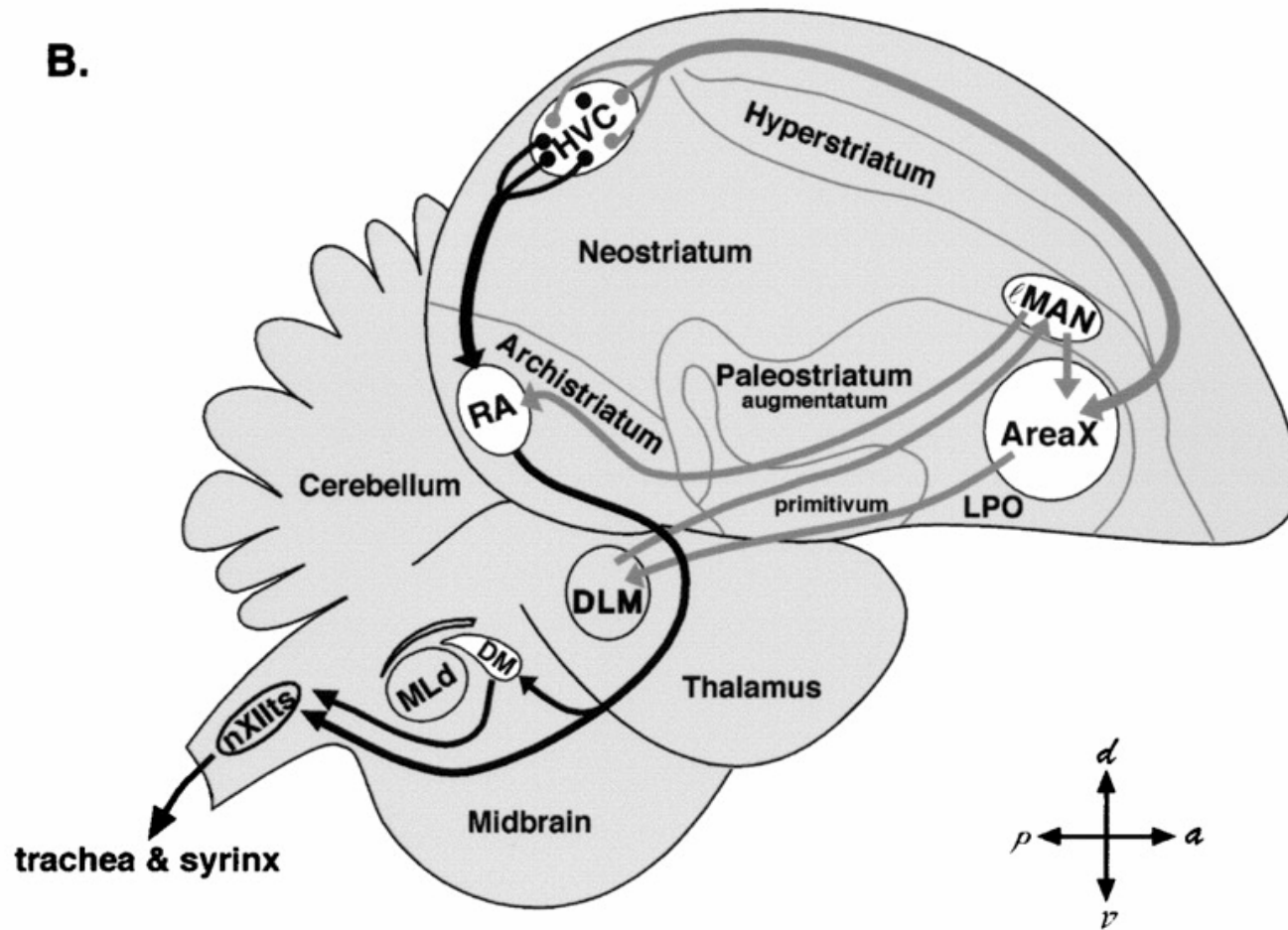
# Role of Auditory Feedback and the Template Theory

Studies of deafened birds by Konishi (1965) suggested that young birds form an internal representation of their tutor's song. Young birds attempt to match their vocalizations to this stored template. One 'holy grail' of bird song research : What is the neural substrate of the template?

Evidence suggests it may lie in lMAN.

# lMAN and the song system

B.



# Clues to the function of lMAN

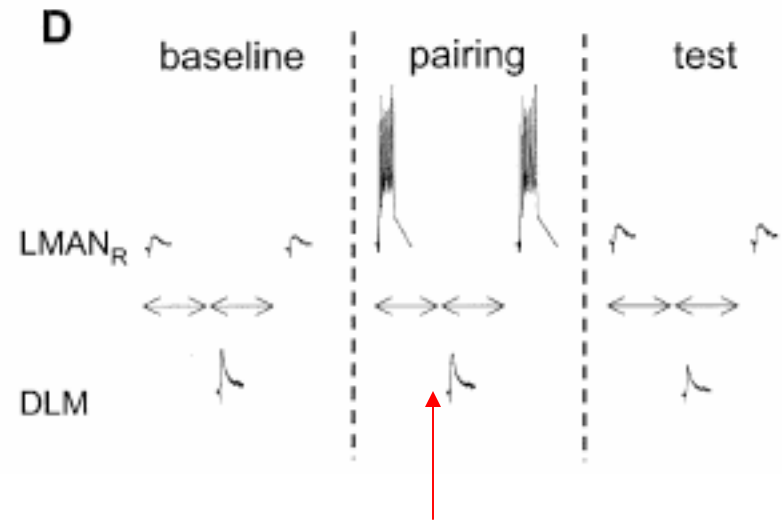
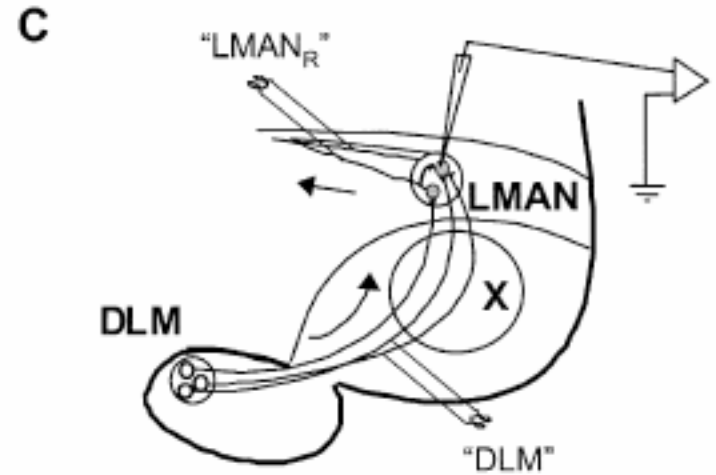
- Lesions to lMAN effect sensory learning as a juvenile but not in adults (Bottjer et al 1984)
- Lesions to lMAN in adults block the natural deterioration following deafening (Brainard & Doupe 2000)
- lMAN is NMDAR rich (Boettiger and Doupe 1998)
- NMDAR expression in lMAN parallels the critical period of sensory learning in juveniles (Aamodt et al. 1992)



# Experimental Methods

- Slice stimulation and recording in current clamp mode.
- lMAN stimulation from DLM paired with depolarizing injections to lMAN neuron.
- Alternating with lMAN stimulation through lMAN recurrent collateral with depolarizing injections to lMAN neuron.
- APV (NMDAR antagonist) infusions.

## IMAN recording and stimulating set-up



(D) Pairing protocol schematic. Brief (100 ms) postsynaptic depolarizing current was delivered 40× in conjunction with LMAN<sub>R</sub> 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.

DLM stimulation followed the depolarizing pulse

## Examples of time intervals between an EPSC input and the peak of a post-synaptic spike.

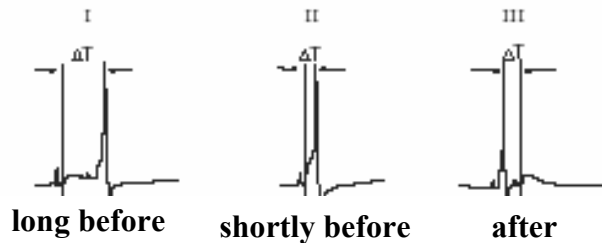
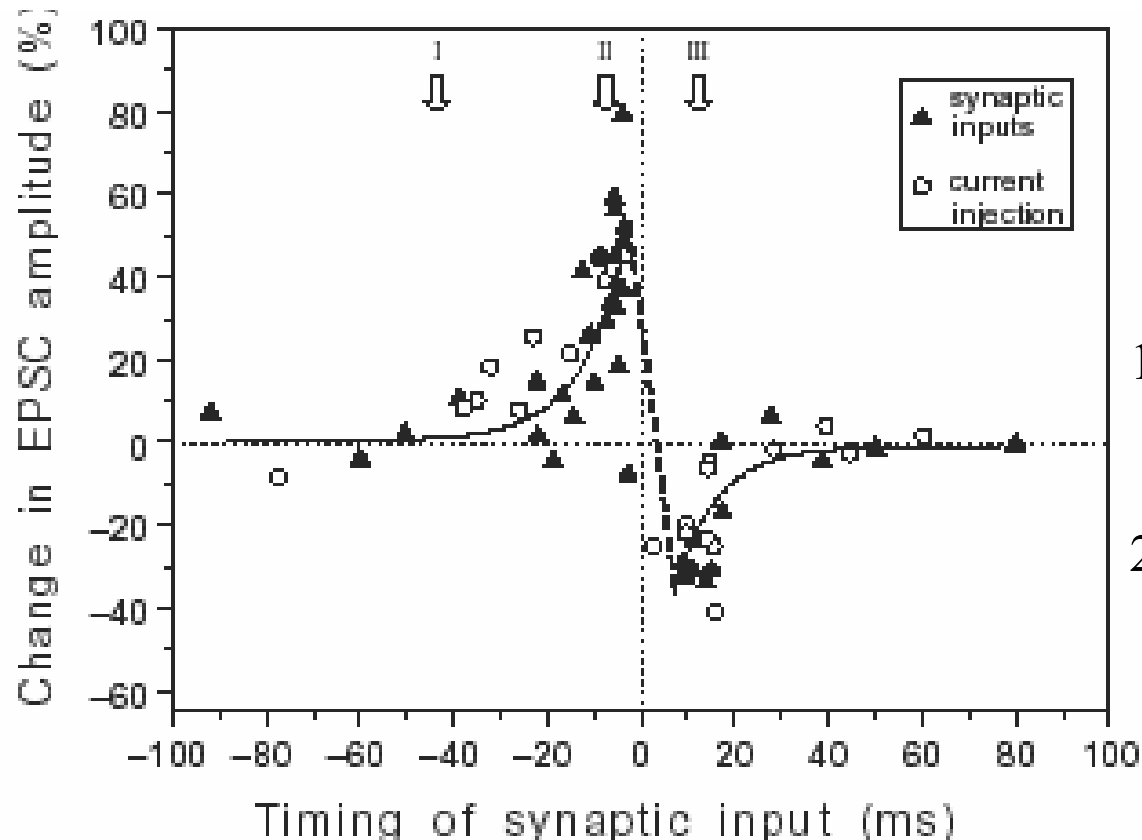


Fig. 2. The critical window for activity-dependent modification of developing retinotectal synapses. The percentage change in the synaptic strength (EPSC amplitude) after repetitive retinal stimulation was plotted against the onset time of the retinal input relative to the peak of the action potential initiated in the tectal cell. Data shown are for experiments in which spiking of the tectal neuron was initiated by either a suprathreshold input or a group of coactive inputs (filled circles), or by injection of a depolarizing current (open circles) (adopted from ref. 69).



## SPIKE-TIMING Mechanism for LTP or LTD

1. Inputs preceding a post-synaptic spike peak by -20 ms or less are reinforced.
2. Inputs following a post-synaptic spike peak by +20 ms or less are depressed,

# Spike Timing Dependence of LTP in lMAN

Postsynaptic  
depolarization precedes  
pre-synaptic stimulation

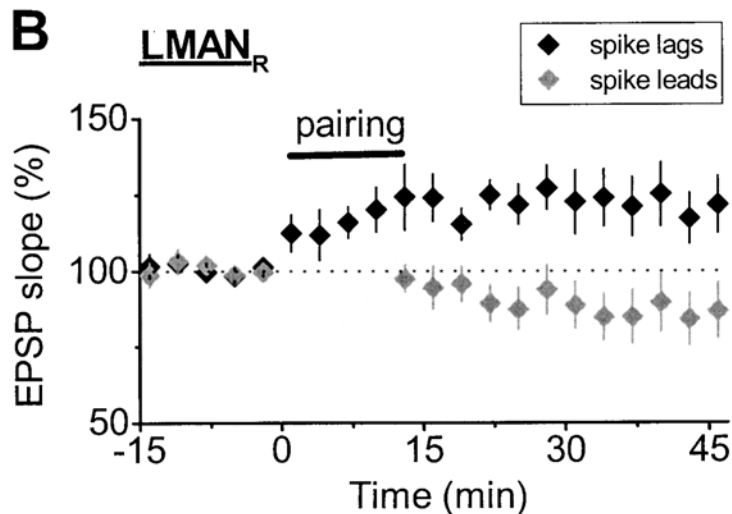
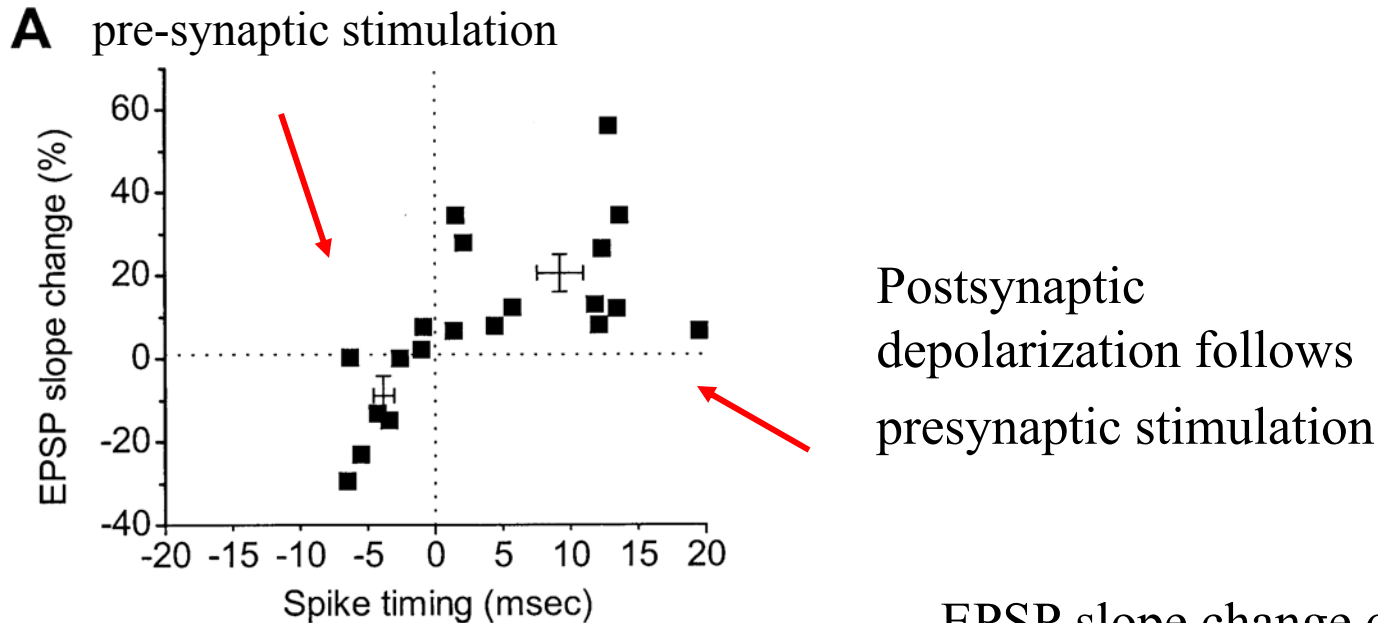
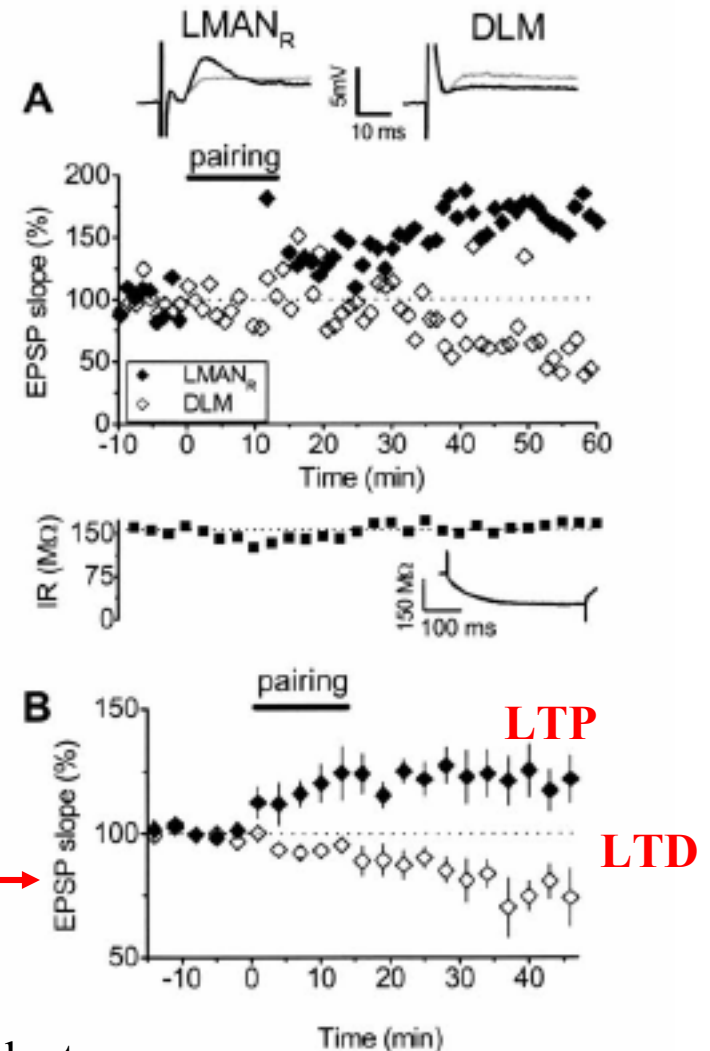


Figure 3

**Figure 2. LMAN<sub>R</sub> 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<sub>R</sub> (◆) 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).



**Averaged results from  
12 LMAN neurons**

Depression in DLM inputs was not dependent on spike timing of DLM to depolarization.

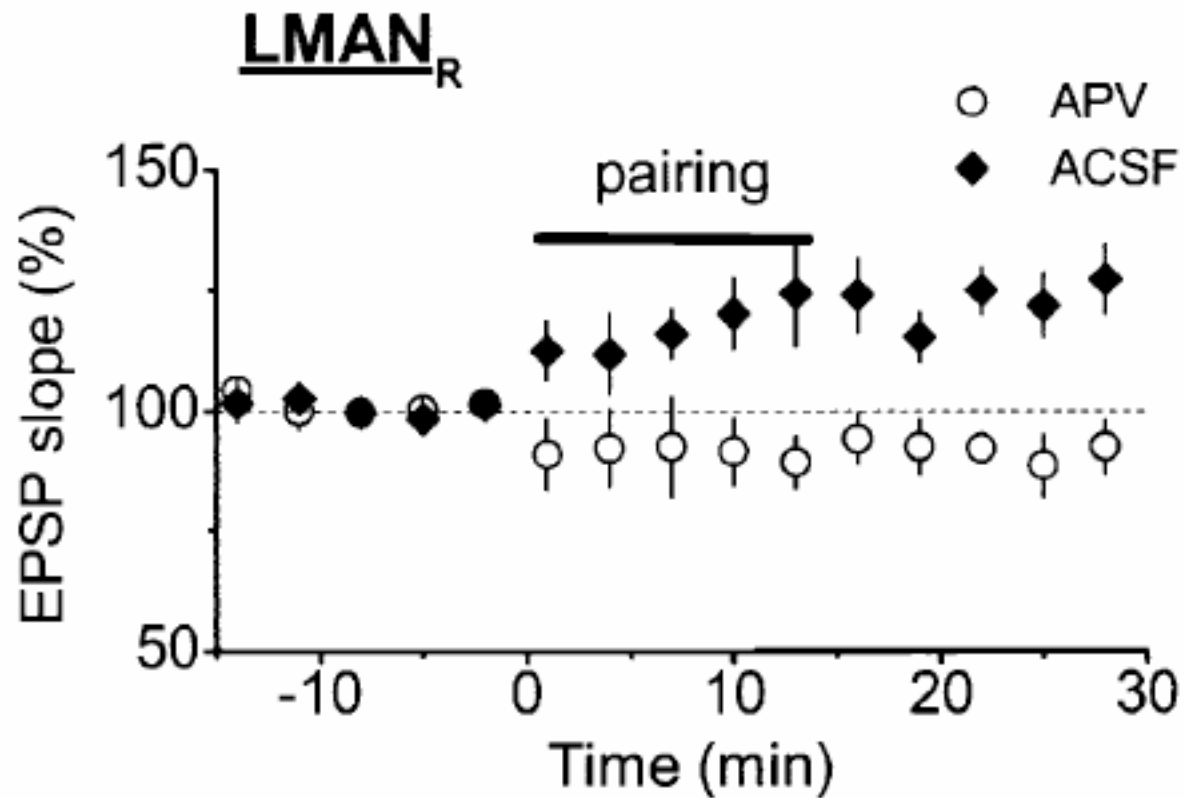
However, the DLM input was not depressed when it occurred anywhere between 15ms before or 15 ms after the lMAN neurons' depolarization induced spiking.

This suggests that the DLM input is not depressed when the DLM is active during lMAN neuron depolarization.

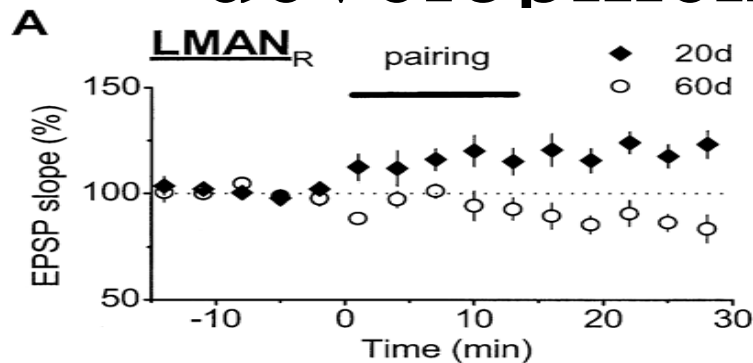
Assuming that DLM depression will eventually lead to the loss of the contact, this time-dependent interaction will successfully prune any DLM inputs that are not closely correlated in time with lMAN depolarization. It is known that DLM inputs to lMAN are pruned during this interval (Johnson and Bottjer, 1992).

These LTD changes are pre-synaptic as both CV and PPR increase with the LTD.

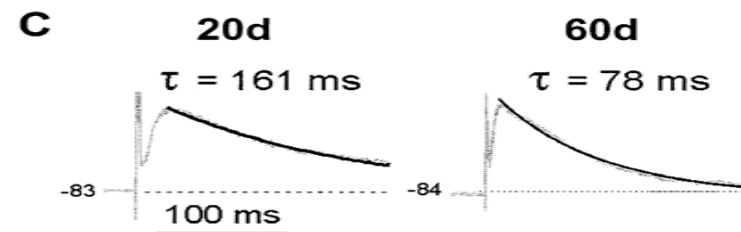
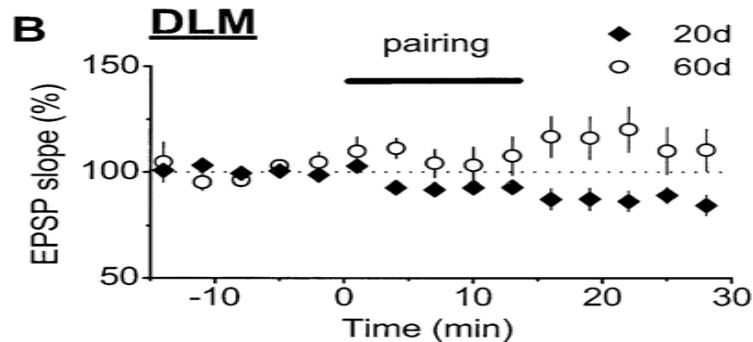
## NMDA receptor blockade eliminates LMAN<sub>R</sub> LTP and Causes LTD in young birds



# LTP/LTD in lMAN is developmentally restricted



A: lMAN. Black diamonds for PHD (post hatching day) 20 slice, white circles for PHD 60 slice.



C: Change in lMAN EPSP time constant. This change is partially due to a decrease in the membrane time constant because lMAN neurons decrease in total surface area during this period. However this does not account for all of the change. Therefore, part of the change is likely due to a molecular change in the NMDAR receptor.



## **Dopamine Receptors: Nearly 80% are in the striatum; coordination of motor input**

Dopaminergic input comes mainly from substantia nigra (in the floor of the midbrain)

Localization is both pre- and post-synaptic

### 5 Types of Dopamine Receptors

D1 (like) includes D1 and D5 activate adenylate cyclase through  $G_s$

D2 (like) includes D3 and D4 inhibit adenylate cyclase and other effectors through  $G_i/G_o$

Drugs binding dopamine receptors are not very specific: clozapine, haloperidol used  
In treatment of psychiatric disorders but mostly we don't know how they work.

# TABLE 10.1 Functions of $\alpha$ Subunits of G Proteins<sup>a</sup>

Class	Member	Some functions
$\alpha_s$ $G_s$	$\alpha_s, \alpha_{olf}$	Stimulate adenylate cyclase, regulate $Ca^{2+}$ channels
$\alpha_i$ $G_i$	$\alpha_{i-1}, \alpha_{i-2}, \alpha_{i-3}, \alpha_o, \alpha_z$	Inhibit adenylate cyclase, regulate $K^+$ and $Ca^{2+}$ channels
$\alpha_t$ $\diagdown$	$\alpha_{gust}, \alpha_{t-1}, \alpha_t^{-2}$	Activate cGMP phosphodiesterase
$\alpha_q$ $G_o$	$\alpha_q, \alpha_{11}, \alpha_{14}, \alpha_{15}, \alpha_{16}$	Activate PLC
$\alpha_{12}$ $\diagup$	$\alpha_{12}, \alpha_{13}$	Regulate $Na^+/K^+$ exchange

<sup>a</sup>Summarized from Neer (1995).

## Functions of the $\beta\gamma$ subunits

Inhibition of adenylate cyclase  
 Stimulation of adenylate cyclase types II and IV (with  $\alpha$ )

Stimulation of phospholipase  $C\beta$   
 Stimulation of  $K^+$  channel  
 Stimulation of  $Ca^{2+}$  channel

Stimulation of phospholipase  $A_2$   
 Stimulation of phosphatidylinositol-3-kinase

## Modifying toxin

Cholera	$G_s$
Pertussis	$G_i$
Cholera and pertussis	
—	
—	