The electrophysiological properties of HVC-RA synaptic transmission in the adult zebra finch *in vivo*

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Abstract: The synaptic connection from high vocal center (HVC) to robust nucleus of the arcopallium (RA) is a pivotal part of vocal motor pathway in songbirds. Electrophysiological properties of HVC-RA synaptic transmission in adult male zebra finch (*Taeniopygia guttata*) in *in vivo* was investigated by using field potential recording method. Following electrical stimulation of HVC, the evoked field potentials recorded in RA were feeble. The results showed that the remarkable paired-pulse facilitation was induced by paired-pulse stimulation at HVC-RA synapses. The results also showed that the evoked field potentials were significantly decreased after a conditioning tetanic stimulation and finally recovered gradually within 15 min, which indicates tetanic stimulation-induced transient depression is present at HVC-RA synaptic transmission. These results suggest that properties of synaptic transmission in this pathway might play a role in controlling song production.

Key words: HVC-RA synaptic transmission; electrophysiological properties; zebra finch (*Taeniopygia guttata*)
eral part of the magnocellular nucleus of the anterior neostriatum (LMAN) that connects to RA. AFP indirectly connects with VMP by a proportion of HVC neurons project to the area X [1-3].

Many aspects of neural mechanisms underlying song learning and production still remain unknown, despite rapid progress in this research field. It is significant to study electrophysiological properties of song nuclei. Usually, electrophysiological properties of synaptic transmission in the song system are investigated using in vitro songbird brain slice preparation. Publications reporting in vivo electrophysiological investigation are scarce. Here, we showed the initial results in examining the electrophysiological properties of HVC-RA synaptic transmission by in vivo field potential recording.

1 MATERIALS AND METHODS

Subjects were 15 male zebra finches (Taeniopygia guttata). Each was anesthetized with an intramuscular injection of urethane (0.5 g/kg) and then placed in a stereotaxic apparatus with a beak bar designed for the zebra finch. The regions of HVC and RA were located according to the zebra finch and canary brain atlas [4].

The nickel-chrome alloy electrode or borosilicate glass microelectrodes filled with 2 mol/L NaAc (resistance of 1~5 MΩ) was used in the in vivo electrophysiological recording. A self-made concentric metal stimulating electrode was positioned into HVC on one side of the brain (AP: 0.0~0.6, L/R: 1.2~2.5, H: 0.2~0.8), then the recording electrode was vertically advanced into RA ipsilaterally (AP: 1.0~1.3, L/R: 2.2~2.5, H: 2.0~2.4), and the evoked field potentials in responding to the HVC stimulations were recorded from the nucleus (Fig.1A).

Stimulus intensities ranged from 5 to 20 V, and single square pulse duration was 0.1 ms. The evoked field potentials were amplified, digitized and averaged for data acquisition and data analysis. In each experiment, an input-output curve was tested firstly before the evoked field potentials were recorded and test stimulus intensity was chosen to 70% equivalent to maximal response. Analyzing method of evoked field potential parameters was shown in Fig.1B. Each data point in the input-output curve is an average of four responses recorded corresponding to each stimulus intensity. The purpose to make input-output curve is to ensure appropriate test stimulus intensity.

Tests of paired-pulse response were conducted immediately after completion of the input-output curve. Paired-pulse stimulation is commonly utilized to test synaptic facilitation effects and consists of two successive pulses that are of equal stimulus intensity at a variety of interpulse intervals. The first pulse is conditioning pulse and the second pulse is test pulse. Interpulse intervals (IPIs) of paired-pulse stimulation used in our experiments were 50, 75, 100 and 150 ms. Twenty pulses were used at 100 Hz as tetanic stimulation and were delivered following a stable baseline recording for 20~30 min. The data were expressed as (mean ± SD) percentage baseline of the responses 20 min prior to tetanic stimulation. Changes in the post-tetanic response were tested and evaluated by comparing the percent change between the pre- and post- responses to tetanic stimulation. Test stimuli were delivered at interval of 30 s. Statistical analysis of two groups was made using t-test.

![Fig. 1. Stimulating and recording place and evoked field potential analysis. A: The stimulating and recording place is illustrated. B: Schematic diagram of evoked field potential analysis. Evoked field potential parameters analyzed included the peak amplitude (mV; a and b) and the peak slope (mV/ms; c and d).](image-url)
2 RESULTS

2.1 Waveform characteristics of evoked field potentials in RA
The evoked field potentials were recorded from RA by stimulating the HVC and were confirmed according to the evaluation that the response latency must be stable and waveform of the evoked potential should not be changeable while stimulation polarity was reversed. The waveforms of the evoked field potentials were changeable in a certain extent. Usually, a few feeble peaks frequently followed the primary peak. The waveform of the evoked field potential changed with increase of stimulus intensity.

Threshold of stimulating voltage for eliciting an evoked potential was (6.029 ±1.537) V. The evoked field potentials were characterized by that the peak latencies ranged from (4.943 ±1.234) to (5.986 ± 1.337) ms, and the peak amplitudes of the evoked potentials ranged from 0 to (0.494 ± 0.198) mV with the peak slope ranged from 0 to (0.926 ± 0.099) mV/ms.

The input-output curves of both amplitude and slope of the evoked potentials were shown in Fig.2.

2.2 Effect of paired-pulse stimulation on HVC-RA synaptic transmission
According to the input-output curve, test stimulus intensity was confirmed. When the paired-pulse stimulation at test stimulus intensity was delivered to HVC, responses in RA were recorded. The remarkable paired-pulse facilitation (PPF) was observed. PPF is known as a phenomenon of short-term synaptic plasticity whereby a second synaptic response is enhanced by a preceding stimulation of similar intensity. Figure 3 showed an example trace of PPF at interval of 50 ms for amplitude. Data were expressed as a ratio of test pulse to conditioning pulse amplitude of potentials, called paired-pulse facilitation ratio (PPFR). A ratio of more than 100% represents PPF, or a ratio of less than 100% reflects paired-pulse depression (PPD). Data of PPFR at IPIs of 50, 75, 100 and 150 ms were (221.33 ± 77.25)%, (196.23 ± 47.58)%, (181.88 ± 45.86)% and (141.68 ± 28.08)%, respectively. As shown in Fig.3, PPFR would be weakened along with increase of IPI. Statistical analysis demonstrated that the PPFR at intervals 50 and 150 ms were significantly different (P<0.01).

2.3 Effect of tetanic stimulation on HVC-RA synaptic transmission
Next, the tetanic stimulation (20 pulses at 100 Hz) was delivered to HVC. Example traces of comparing post-tetanic waveforms with pre-tetanic waveforms were shown in Fig.4. In these experiments, tetanic stimulation induced a phenomenon of transient depression (less than 15 min) in HVC-RA synaptic transmission. Statistical analysis demonstrated that the evoked field potentials were significantly weakened after tetanic stimulation and recovered gradually within 15 min (Fig.4B and C).

To explore the effect of tetanic stimulation on HVC-RA
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In *in vivo* synaptic transmission further, we compared the PPFR of the evoked field potentials before and after the tetanic stimulation. Paired-pulse stimulation of IPI at 50 ms was used as test stimulation. Statistical data indicated that the PPFR post tetanic stimulation within 8 min are significantly less than the PPFR prior to tetanic stimulation, as shown in Fig. 4D (P<0.05).

**3 DISCUSSION**

The song control pathway in the forebrain is responsible for song production and learning in songbirds. Male zebra finches imitate a tutor song after hatching and retain it with little change for the rest of their lives [2,5].

Vocal learning pathway has been shown to produce long-term potentiation (LTP) of synaptic efficacy in brain slice preparations *in vitro* [6]. We investigated the electrophysiological properties of HVC-RA synaptic transmission in adult male zebra finch VMP using *in vivo* field potential recording, and found that: (1) paired-pulse responses are relevant to modulating cell excitability [9] and have some functional significance in motor control [10].

Tetanic stimulation is used to test synaptic plasticity. The phenomenon of transient depression after tetanic stimulation.

It has been considered that PPF is produced by the build-up of calcium in the synaptic terminal and is dependent on the transmitter release probability during presynaptic activity, a presynaptically mediated phenomenon, and on the post-synaptic effect of ligand of the receptor [7]. An example of a PPF synapse is the parallel fiber inputs to Purkinje cell in the rat cerebellar cortex. The PPF typically lasts for several hundred milliseconds following presynaptic activity [8]. It is probable that paired-pulse responses are relevant to modulating cell excitability [9] and have some functional significance in motor control [10].

Tetanic stimulation is used to test synaptic plasticity. The phenomenon of transient depression after tetanic stimulation.

**Fig. 3.** Paired-pulse facilitation and paired-pulse facilitation ratio. A: An example trace showed paired-pulse facilitation at interpulse interval of 50 ms in HVC-RA pathway. B: Average paired-pulse facilitation ratios with increase of interpulse intervals (*n*=15 for data points at interpulse intervals of 50, 75 and 100 ms; *n*=5 for data point at interpulse interval of 150 ms). Error bars indicate SD.

**Fig. 4.** The effects of tetanic stimulation on HVC-RA synaptic transmission. A: Representative traces from a single experiment of evoked field potentials 2 min before the tetanic stimulation, 2 min, and 12 min after the tetanic stimulation respectively. B, C: Changes in the amplitude and slope of evoked field potentials following a tetanic stimulation (20 pulses at 100 Hz) (*n*=12). Each data point is a 2-minute average. Baseline recordings were conducted for at least 20 min prior to applying a tetanic stimulus (“T”) to HVC. D: Changes of paired-pulse facilitation ratio following a tetanic stimulation (“T”). Each data point is a 2-minute average PPR value (*n*=5). Error bars indicate SD.
transmission elicited by tetanic stimulation might attribute to transmitter exhaustion or probability of release decrease\cite{11,13}. During the period of depression, the PPFR was significantly diminished, which indicated that the transmitter release probability was decreased. This result further suggested that the depression might be correlated with presynaptic release probability decrease. Other studies in brain slices demonstrated that action potential firing rate of the most of neurons in the HVC would exceed 100 Hz on the condition that stimulus intensity was appropriate\cite{14,15}. So, it is probable that the transient depression evoked by tetanic stimulation has physiological significance in HVC-RA pathway.

Formerly known as the high vocal center in songbirds, HVC is anatomically termed the caudal nucleus of the hyperstriatum ventral and project fibers to RA to constitute the major VMP. Synaptic interactions within the HVC are implicated in motor and auditory activity associated with learned vocalizations\cite{10}. The HVC affords a site for auditory-vocal integration and is the probable source of auditory input to other nuclei important to song production, development, and perception\cite{17}. It has been considered that the HVC plays a major role in the motor profile of song production, especially in the programming of time sequences of song components\cite{18,19}. Comparing with the studies in some motor pathways of other animals\cite{10}, we speculated that short-term changes of synaptic efficacy in HVC-RA pathway might play a role in motor control of songbird vocalization.

According to histological observation, the neurons in RA are dispersed in small clusters. The characteristic of dispersed neurons is probably the main reason why the evoked field potentials in vivo were faintly recorded and difficult to stable using in vivo field potential recording. Further studies to investigate changes of vocal behavior and pharmacological mechanisms underlying synaptic plasticity in HVC-RA pathway will contribute to understanding the mechanisms of song production in songbirds.

REFERENCES

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