SONG INDUCED SPECIFIC CARDIAC RESPONSES ARE MODIFIED BY THE PARTIAL LESION OF THE HIGHER AUDITORY AREAS (NCM) IN BENGALESE FINCHES

PACS REFERENCE: 43.80.Lb., 43.80.Ka

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ABSTRACT

The NCM, a region of the higher auditory area in avian telencephalon, is a site of gene expression when stimulated with conspecific songs. We used cardiac response to characterize the process related to song perception in Bengalese finches. Song playback evoked an increased heart rate, but repeated exposure to one song resulted in habituation. Habituation did not occur, however, when exposed to series of unique songs in females, but not in males. Lesioning NCM in females completely abolished this sex specific cardiac response. This is the first demonstration that the NCM is involved in auditory memory at behavioral level.

INTRODUCTION

In many passerine songbirds, males sing to defend a territory and to attract females and females, on the other hand, listen to the songs to assess the quality of male [1][2]. In a previous study we have described the experiment that measured heart rate responses to conspecific and hetero-specific song presentations [3]. When novel conspecific songs were presented, female Bengalese finches reacted by increasing the heart rate but males did not react in such ways. Results matches with ecological specificity of this species. In Bengalese finches, songs are solely used in mating context by males to attract females. They never use songs in aggressive contexts.

Functions of the NCM had been studied at the cellular level and suggested to be responsible for the auditory memory formation. However, this idea had never studied at the level of individual birds. We utilized heart rate responses that were successful in describing species specific memory processes in Bengalese finches to study the relationship between memory processes and the NCM function.

METHODS

Animals Birds were obtained from local pet suppliers. Eight adult female and four adult male Bengalese finches were used. These twelve birds received the NCM lesion. Seven adult female and three adult male Bengalese finches were used as control. Some of the control birds received shame operations while other were kept intact. These cages were kept in a controlled environment (temperature 25., relative humidity 60%, 14L10D day cycle) in the aviary at Chiba University with some estrildid species.

Stimuli Seven Bengalese finch songs and seven zebra finch songs were selected randomly from a library of recordings in our laboratory. No songs recorded from subject birds were selected. To avoid using similar songs, no two songs were selected from one family. Songs were recorded with a digital audio tape recorder (SONY, DTC-ZA5ES) using an electric condenser microphone (SONY, ECM-MS957). Recorded songs were edited using sound analysis software (SAS Lab ver 3.0, Avisoft, Inc.); the total duration of a song was between 1.6 and 3.2 sec. The songs were digitized at 22.1 kHz and stored on a hard disk. Songs were played back during the experiment using an analogue-to-digital converter (Data Translation, DT2801), amplified, and broadcast by a magnetically shielded speaker.

Cardiac Recording The method of electrode insertion (Fig. 1a) and the apparatus used for the recording (Fig.1b) Recording time were 75 seconds, 10 seconds before stimuli and 65 seconds after stimulus (Fig.2).

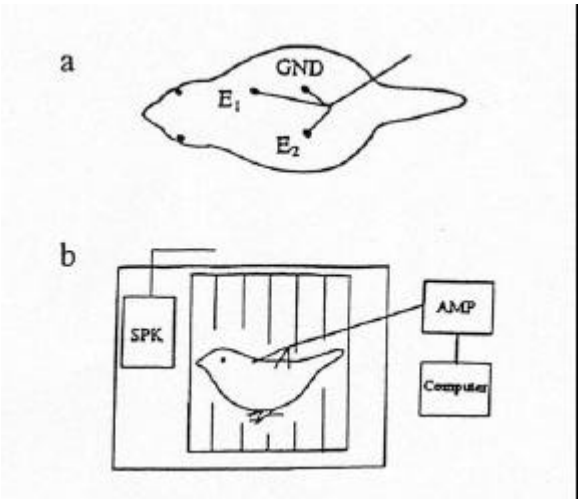

Fig.1a Positions of the recording electrodes. Electrodes were inserted into 3 points on the bird's back. E1 was a positive electrode, E2 negative, and GND was the grand electrode. The tip of a No. 00 insect pin was modified to form a hook shape and inserted under the skin. Output of these were connected with a connector that was attached by a surgical tape on the bird's back.

b. Schematic diagram of the experimental setup.
The bird was placed inside a dark sound proof, magnetic shielded chamber (60.60. 80cm). Cardiogram was amplified by the biological amplifier and conditioned by the bandpass filter, then analyzed by an electrophysiology software.

Procedure There were 24 trials in on experiment. The stimulus songs were divided into 4 categories. These were (a) a Bengalese song that was presented a total of 6 times within a session (Bengalese repeat; BR), (b) a Zebra finch song that was similarly presented 6 times (Zebra repeat; ZR), (c) four Bengalese finch songs (Bengalese single; BS; n = 1to 6), and (d) four zebra finch songs (Zebra single; ZS; n= 1 to 6) that were each presented only once during a session. Thus, a block of 4 trials consisted of BR, ZR, BS and ZS, and there were 6 blocks per session. The order of presentation within a block was randomized, and a unique random order was used for each bird.

Quantitative Measurement of Cardiac Response Methods for evaluating the heart rate responses were described elsewhere [3]. A typical time course for the heart rate before and after song stimulation in the pilot study for a Bengalese finch is shown in Fig. 2. For each trial, cardiac data was recorded during the 10-sec interval before stimulus presentation. An average heart rate for this 10-sec interval was obtained and the average and standard deviation of 24 such measurements were calculated. We drew a line 2 standard deviations above the grand average (AV_{24}+ $2SD_{24}$), and measured the length of time that the heart rate exceeded the line during and after song playback (Fig. 2). This period was defined as the duration of the cardiac rate increase (DCRI).

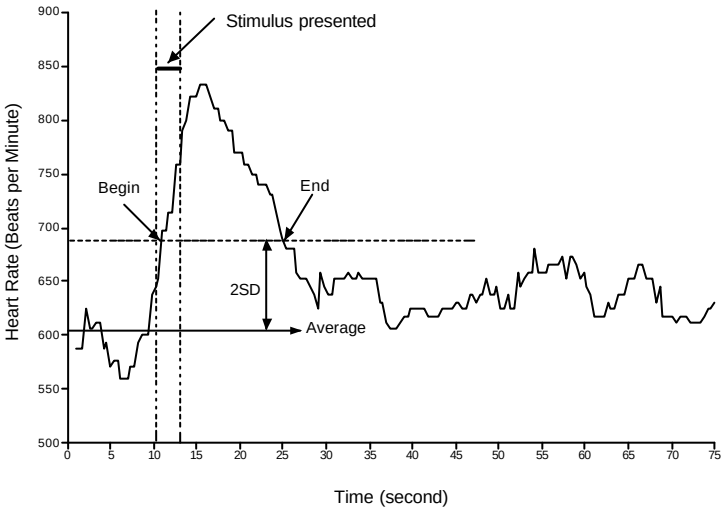

Fig.2 Heart rate change before and after stimulus presentation. An example of heart-rate change before and after a song presentation. The baseline heart rate was calculated from the interval ten seconds before stimulus presentation. The DCRI refers to the interval between “Begin” and “End”.

RESULTS

Control birds

Both males and females showed increased hear rate for the first couple of stimulus presentations regardless of the stimulus categories. For males, the cardiac response habituated to all categories of stimuli afterwards. Females showed a similar trend except when conspecific novel stimulus was presented. For each unique presentation of conspecific novel song, females increased heart rate. This tendency was the same as previous experiment [3].

NCM Lesioned birds

DCRI for each stimulus presentation was shown for a male and a female as examples. In males, there were no differences between the controls and the lesioned individuals in response tendencies. However, in females, the characteristic response pattern to the novel conspecific songs disappeared when the NCM was lesioned.

Effects of the NCM lesion

Fig. 3 and 4 summarize the later half of the session. In males, there was no difference among stimulus categories in the control group. In the NCM lesioned males, singly presented zebra finch male songs induced longer DCRI but difference was not significant. When the two groups were compared by the two-way ANOVA followed post hoc comparisons, the two groups were not significantly different on any of the stimuli (Fig. 3). In females, the specific effect on the conspecific, unique stimuli totally disappeared by the NCM lesioning operation. By one-way ANOVA, in the control females only BS stimuli induced significantly longer DCRI than other stimuli ($F=13.0$, $df=3,15$, $p=0.0002$)(Fig. 4). For the last half of the session, two-way ANOVA detected significant effect of stimulus ($F=4.39$, $df=3, 52$, $p=0.0079$) and interaction ($F= 4.01$, $df=3, 52$, $p=0.0121$). By the post-hoc comparison, the control group and the lesion group was significantly different only when compared on the BS stimuli ($t=3.79$, $p<0.01$)(Fig. 4).

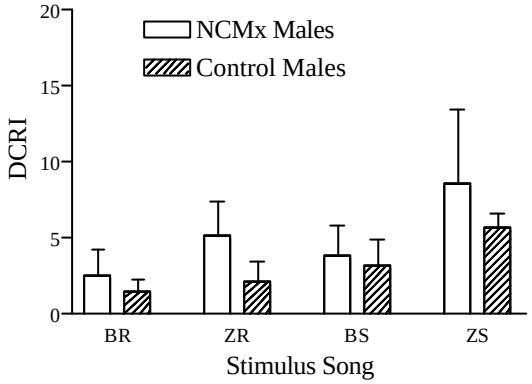

Fig.3 Average heart-rate increase for each category of stimuli taken from the last half of the session for males. There was no difference between the control and the lesioned males for any of stimulus.

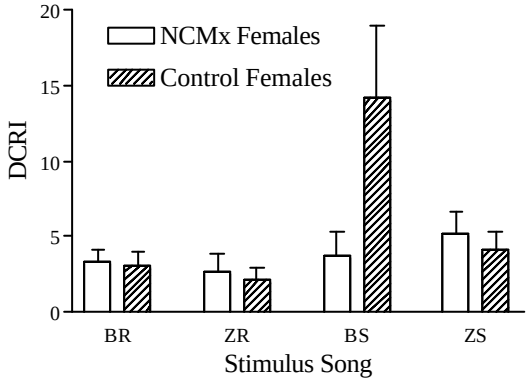

Fig.4 Average heart-rate increase for each category of stimuli taken from the last half of the session females. The control and the lesioned females were different for the BS stimulus.

DISCUSSION

This is a first attempt aimed at extending the functions of the NCM from the cellular level to the level of whole animal. The NCM shows hearing-induced gene expression when stimulated with conspecific vocalizations. Since the patterns of IEG expression showed novelty-detector like properties, the NCM was considered to be responsible for auditory memory processes. There was no support for this hypothesis at the level of individual behavior until now. When the NCM was partially lesioned in female birds, the specific pattern of cardiac response disappeared suggesting that the NCM was in fact responsible to determine whether the song was novel or not.

Perception of females and the NCM

The control females showed the longer duration of increased heart rate to the presentation of unique conspecific songs. Results show untreated females distinguish between individual, conspecific songs and react by increasing hear rate to novel conspecific songs. This suggests that females are able to discriminate among different conspecific songs more accurately. This ability, in turn, should be adaptive for females to select more superior males [1][2].

However, when the NCM was partially, but bilaterally lesioned in females, the specific reaction to the novel conspecific songs disappeared completely (Fig. 4). By lesioning the NCM, females failed to distinguish between the novel conspecific songs and familiar songs. The NCM, therefore may play a critical role for females in deciding on which mate to chose.

Neurophysiological and molecular histochemical studies by Mello et al. [4], Chew et al. [5], Chew et al. [6] and Riberio et al. [7] all suggested that the NCM must be functioning as a novelty filter for conspecific songs at a celluler level. The present study showed, for the first time, the NCM indeed functions as a novelty filter at the level of individual bird.

Perception of males and the NCM

Compared to the females, the NCM lesion experiment did not change any of males' cardiac reaction. We simply can not discuss about memory processes in males based on the present results. Regardless of the NCM lesion or not, all males reacted only for the first 2-3 presentations of songs.

By cellular levels of analyses, the pattern of gene expression and electrophysiological responses showed properties suitable as the novelty detector for conspecific vocalizations in both females and males. However, as shown by my research, when heart rate response was taken as the index, even the control males did not show any response to the novel conspecific songs, unlike the control females.

Therefore, we do need other behavioral assay to detect the function of the NCM in male birds. One possible solution to this problem may be to consider for the ecological need. In species that defend territory by songs heart rate response was detectable in male birds [8]. If we used these species, we might be able to show the novelty response. In such species, we will be able to show the effects of the NCM lesion at the individual level.

CONCLUSION

We were able to extend the function of the NCM to the level of individual. Before our research, the notion was limited only to the cellular level. Research on memory should not remain at the cellular level, since the memory belongs to individual animal, not to the individual cells. By relating cellular level of knowledge with individual level of analyses, we will be able to gain more insight on the mechanisms of memory. The NCM and auditory memory in birds will be an ideal subject for such studies.

ACKNOWLEDGMENTS

We thank Dr. Nozomu Saito in Dokkyo Medical University for instructing me with the cardiac recording procedures, Miho Futamatsu for the help provided in the cardiac recording experiments, and Tikara Hoshino for programming the cardiac recording system. Financial support for MI was provided by JSPS. Work supported by *PRESTO*, JST and by JSPS.

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