Social interaction with a tutor modulates responsiveness of specific auditory neurons in juvenile zebra finches

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ABSTRACT

Behavioral states of animals, such as observing the behavior of a conspecific, modify signal perception and/or sensations that influence state-dependent higher cognitive behavior, such as learning. Recent studies have shown that neuronal responsiveness to sensory signals is modified when animals are engaged in social interactions with others or in locomotor activities. However, how these changes produce state-dependent differences in higher cognitive function is still largely unknown. Zebra finches, which have served as the premier songbird model, learn to sing from early auditory experiences with tutors. They also learn from playback of recorded songs however, learning can be greatly improved when song models are provided through social communication with tutors (Eales, 1989; Chen et al., 2016). Recently we found a subset of neurons in the higher-level auditory cortex of juvenile zebra finches that exhibit highly selective auditory responses to the tutor song after song learning, suggesting an auditory memory trace of the tutor song (Yanagihara and Yazaki-Sugiyama, 2016). Here we show that auditory responses of these selective neurons became greater when juveniles were paired with their tutors, while responses of non-selective neurons did not change. These results suggest that social interaction modulates cortical activity and might function in state-dependent song learning.

1. Introduction

Perception, and even sensation, can vary from moment to moment depending on an animal's behavioral state or context. The same acoustical signals can be sensed differently when attention is focused on them. Interestingly, focused attention modulates sensing or recognition of a specific signal, rather than shifting overall sensitivity. This suggests that behavioral states can filter sensory signals to target them for further processing. Recent studies have shown that the behavioral state directly modifies cortical sensory responsiveness via top-down cortico-cortical input, rather than through thalamic feedforward inputs. Neuronal responses to visual stimulation in V1 neurons of awake mice, but not in neurons of the lateral geniculate nucleus (LGN), increased during locomotion (Niell and Stryker, 2010). Locomotor activity also decreased auditory responses in A1 by top-down M1 input through local inhibitory circuits (Schneider et al., 2014). Sensory responses are not just increased or decreased, rather response tuning can also be modified when animals are engaged in a task (Fritz et al., 2003).

Songbirds learn to sing through vocal communications during early development. In zebra finches, which are highly social and less

territorial, and have long-lasting pair bonds, juvenile song learning is greatly improved through social vocal communications, although juveniles can learn from playback of recorded tutor songs (Bolhuis et al., 2000), much as human infants do not learn phonetic detection in speech via audio-visual aids (Eales, 1989; Kuhl 2010; Chen et al., 2016). Interestingly, juvenile zebra finches do better at learning from song playback when they are engaged in juvenile task performance (Tchernichovski et al., 2001). They also learn more from conspecific adults with which they interact intensely when they have multiple choices (Williams, 1990). Those studies suggest that juvenile behavioral states, such as focused attention during social interaction, filter out background auditory signals, so that tutor song forms exclusive memories.

Various studies have shown that auditory memories of tutor song are stored in the zebra finch caudomedial nidopallium (NCM), homologous to the mammalian higher auditory cortex. Greater numbers of neurons in the NCM manifest immediate early gene expression in birds exposed to tutor songs, than in birds exposed to novel conspecific songs (Gobes et al., 2010). The expression level of immediate early genes in birds that are exposed to a tutor song is proportional to the amount of
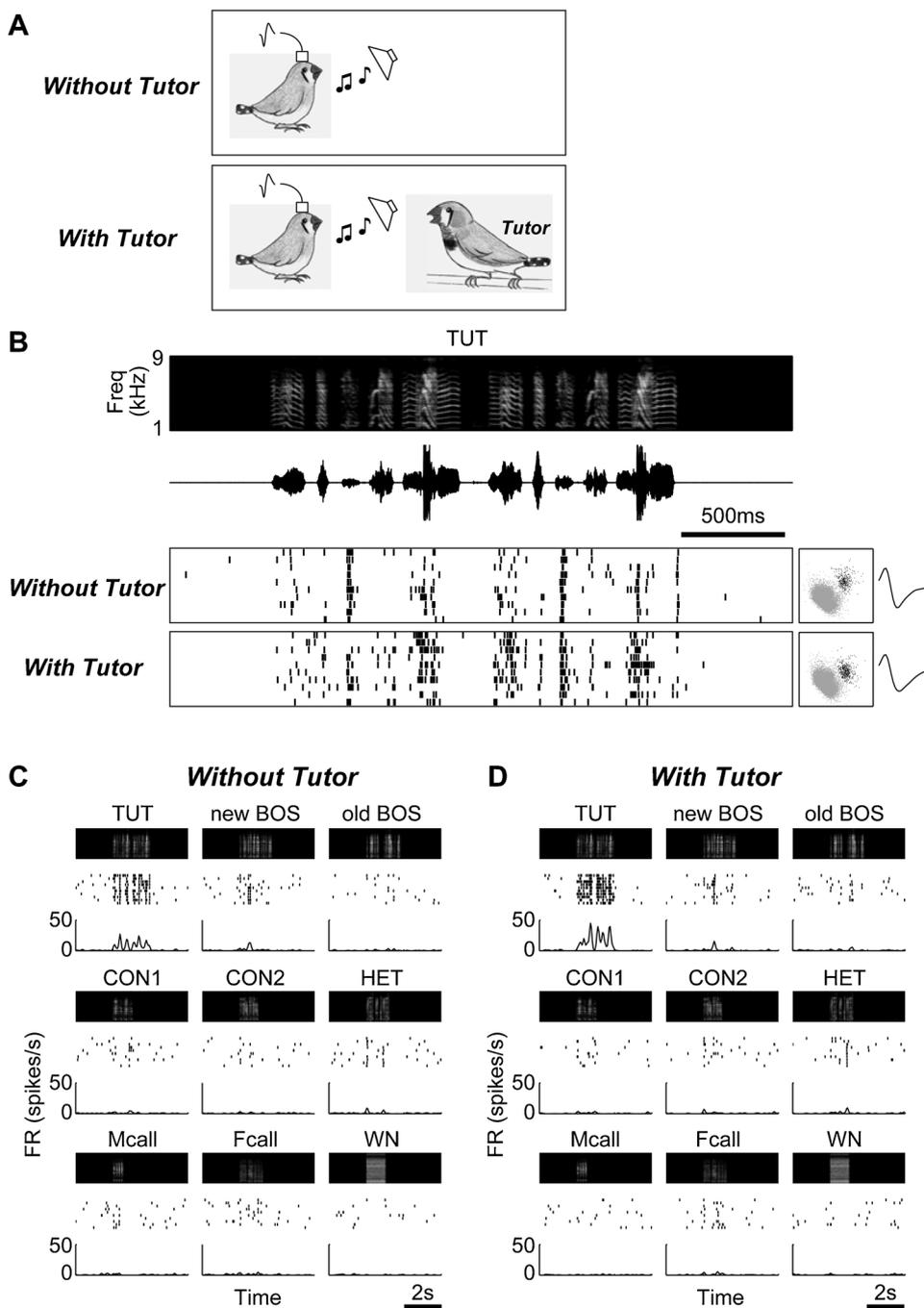
Fig. 1. Tutor presence modifies auditory responses in the zebra finch auditory cortex.

A: Diagram of the experimental design. Neuronal responses to various song stimuli were recorded in juvenile zebra finch higher auditory cortex in freely behaving conditions, with or without a tutor present. B–D: With the tutor present, neurons in the higher auditory cortex, the NCM, show greater responses to song stimuli. B: Representative responses to a tutor song (TUT)-selective neuron with and without the tutor. Sonogram (top), oscillogram (middle), and raster plots (bottom) are shown. Spike clusters and spike waveforms with or without the tutor are shown on the right. C–D: Auditory responses of the same TUT-selective neuron without (C) and with (D) a tutor. Sonogram (top), raster plots (middle), and firing rate histogram (bottom) for each sound stimulus are shown.

tutor song learning (Bolhuis et al., 2000). Electrophysiological recording shows faster habituation with repeated tutor song presentation than with repeated novel songs (Phan et al., 2006). Pharmacological blockade of ERK activation during tutor song exposure prevents juveniles from learning these songs (London & Clayton, 2008). Recently we suggested that early tutor auditory experiences shape cortical neuronal circuits to form memories of tutor songs (Yanagihara and Yazaki-Sugiyama, 2016). As a neurophysiological representation of tutor song memory, a specific type of neuron responds selectively to tutor songs after experiencing those songs, and selectivity changes depending on the bird's arousal state. Here we show that these selective neurons, but not non-selective neurons, exhibit greater auditory responses in the presence of a live tutor.

2. Methods

2.1. Subjects and experimental design

Experiments were performed in accordance with experimental protocols approved by the Animal Care Committee at Okinawa Institute of Science and Technology (OIST) Graduate University. Five male juvenile zebra finches, born and reared in our colony, were used in experiments. Birds were raised in cages with their parents and siblings. Then, at 53–62 days post hatch (DPH), male juveniles underwent surgery for electrode implantation under isoflurane anesthesia. After electrode implantation, juveniles were caged singly in sound attenuation chambers, and were occasionally moved to an experimental chamber for electrophysiological recordings. Single-unit activity was recorded from juveniles while they were isolated in the recording

chamber, either by themselves or with a tutor, in order to determine auditory responsiveness to song stimuli.

2.2. Electrophysiological recordings, song stimulation, and data analysis

The surgical procedures, electrophysiological recording methods, song stimulation, and data analysis have been described in detail previously (Yanagihara and Yazaki-Sugiyama, 2016). Briefly, we recorded single-unit neuronal activity from the NCM in freely behaving male juvenile zebra finches (54 ~75 DPH) implanted with tetrode wire electrodes. In the recording chamber, juvenile birds were introduced to song stimuli, which included a tutor song (TUT), the old bird's own song (BOS) (recorded 8.8 ± 5.3 days before the electrophysiological recording day), a new BOS (recorded at 6.0 ± 4.5 days before the electrophysiological recording day), two different conspecific adult zebra finch songs (CON1, CON2), a heterospecific adult Bengalese finch song (HET), an adult male zebra finch call (Mcall), an adult female zebra finch call (Fcall), and white noise (WN). Stimuli were presented from a loudspeaker in semi-random order. Once neuronal responses to song stimuli were recorded, the tutors (fathers) of juveniles were introduced to the isolation chamber. At 1 min after tutor introduction, the same series of song stimuli were introduced and neuronal responses to song stimuli in the presence of the tutor were recorded. Birds were constantly monitored with a video camera during electrophysiological recordings. Auditory neuronal responses of each neuron to song stimuli with or without tutor presence were quantified as response strength (RS). Neurons that showed a significant response (a significant increase in firing rate compared to the pre-song period, $p < 0.05$, paired t-test) when juvenile birds were alone were further analyzed. We assessed response bias to classify selective neurons and to calculate the selectivity index as previously described (Yanagihara and Yazaki-Sugiyama, 2016). After electrophysiological recordings, juvenile birds were sacrificed, and recording sites were identified histologically post-hoc to confirm that all recordings were performed on the medio-dorsal side of the NCM (0.8–1.9 mm depth)

3. Results

We made chronic recordings of neuronal activity of the higher auditory cortex, the NCM, in five freely behaving juvenile zebra finches during the song-learning period (54–75 DPH) and tested auditory responsiveness of these neurons with and without the tutor present (Fig. 1A). We recorded 109 NCM neurons and identified 88 song-responsive neurons that were classified as narrow spiking (NS, n = 34) or broad spiking (BS, n = 54) neurons, with distinctive spike shapes, as previously defined (detailed methods in Yanagihara & Yazaki-Sugiyama 2016). As previously reported, while no NS neurons showed selective auditory responses to any song stimuli, ten out of 54 BS neurons showed selective responses to either the birds' own songs (BOS) (n = 4 from 3 birds) or to tutor songs (TUT) (n = 5 from 2 birds) or both (n = 1) (Fig. 1C). We found another seven neurons that selectively responded to one of the conspecific songs (n = 5 from 4 birds) or calls (n = 2 from 2 birds).

Tutor introduction to the recording chamber did not induce juvenile vocalization in response to song playback. It did not change the spontaneous firing rate (non-selective BS neurons: alone = 2.1 ± 1.7 Hz, with TUT = 2.1 ± 2.4 Hz, p > 0.05, NS neurons: alone = 17.0 ± 11.0 Hz, with TUT = 18.7 ± 11.2 Hz, p > 0.05, Wilcoxon signed-rank test) or response strength to either song stimulus in either NS or non-selective BS neurons (Fig. 2C). Those neurons responded equally well to many song stimuli in the presence or absence of a tutor, indicating that they have low selectivity (Fig. 3). Consistent neuronal responses with or without tutor presence were also observed in selective BS neurons other than TUT- or BOS-selective neurons (five CON-selective neurons, one Mcall-selective and one Fcall-selective). In contrast, in the TUT- or BOS-selective BS neurons, tutor presence
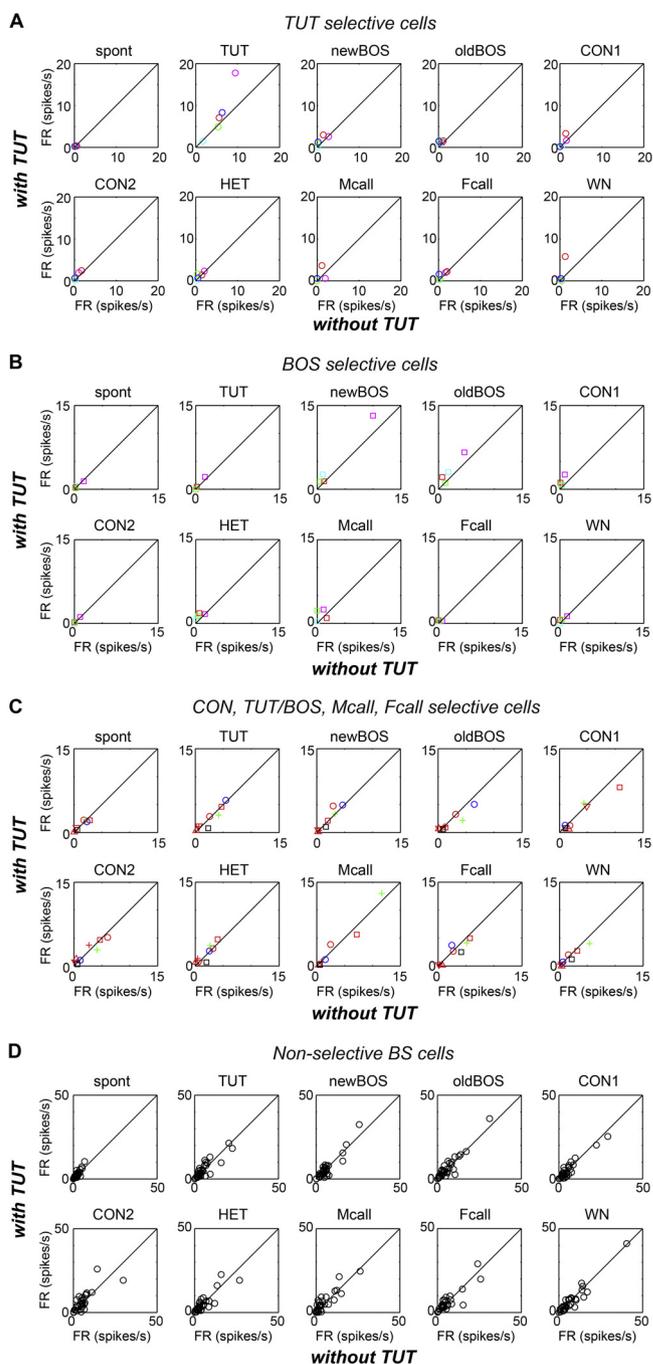

Fig. 2. Selective neurons, but not non-selective neurons, increase their firing rates in response to song stimuli in the presence of the tutor.
Plots of firing rate in TUT-selective (A), BOS-selective (B), CON-, TUT/BOS-, Mcall-, Fcall-selective (C) and non-selective (D) BS neurons during each song stimulus when juveniles were alone (x) and with their tutors (y). Both TUT- and BOS-selective neurons show greater firing rates for most song stimuli, except white noise (WN), when juveniles are paired with their tutors, while non-selective neurons did not change their firing rates in response to any stimuli. Each symbol represents data from a single-unit. Data from the same units are shown with the same color and symbol (circle, square, triangle, plus). In the panel **C**, each color indicates the selective neurons to each song stimuli; red: CON-selective, blue: TUT/BOS-selective, green: Mcall-selective, black: Fcall-selective. 'spont': spontaneous firing rate (FR) during the quiet period. 'TUT': FR during TUT playback.

likewise failed to alter spontaneous firing rate (TUT-selective BS neurons: alone = 0.3 ± 0.2 Hz, with TUT = 0.3 ± 0.1 Hz, p > 0.05, BOS-selective BS neurons: alone = 0.6 ± 0.7 Hz, with TUT = 0.6 ±
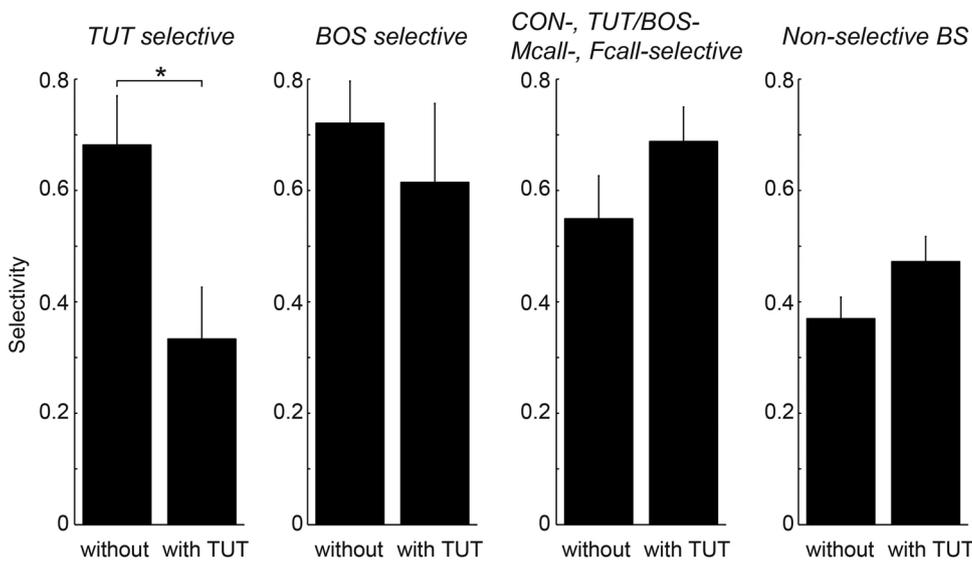
Fig. 3. Tutor presence decreases the selectivity of auditory responses in NCM neurons.

Song selectivity in auditory responses of TUT-selective neurons decreased significantly when juveniles were paired with their tutors (p < 0.05, n = 5, Wilcoxon signed-rank test), while selectivity did not change in BOS-selective (n = 4), CON-, TUT/BOS-, Mcall-, Fcall-selective (n = 8) or non-selective BS neurons (n = 31).

0.6 Hz, p > 0.05); however, it did increase their auditory responses (Fig. 1).Unchanging spike shapes confirmed that the same neurons were recorded before and after tutor introduction (Fig. 1B).

Although tutor presence alters responsiveness of TUT-selective neurons, enhancement of responses was not specific to the TUT alone. Three out of five TUT-selective neurons showed greater responses to the TUT in the presence of the tutor. In addition, they began to respond to other song stimuli to which they had not responded when no tutor was present. The other two TUT-selective neurons did not modify their responses to TUT, but they increased responses to other song stimuli during the tutor was present (Figs. 2 & 3), while a response bias was retained to the TUT. Interestingly the neurons showing greater response to TUT without a tutor presence increased responses more with a tutor introduction. The tutors did not sing songs while they were together with juveniles in the recording chamber.

Similar trends were observed in BOS-selective neurons. All four neurons increased their firing in response to either the old or new BOS, or to both and began to fire in response to other song stimuli when the tutor was present, while retaining a response bias to either the new or old BOS. They exhibited greater responses while decreasing the song selectivity of selective neurons in the presence of the tutor. We did not find any neurons that were unresponsive to all song stimuli in the absence of the tutor, but that began to respond following tutor introduction.

4. Discussion

The tutor presence modifies auditory responsiveness of TUT- and BOS- selective neurons in the juvenile higher auditory cortex, the NCM. However, we could not determine whether this response modification is specific to the presence of the tutor or random another zebra finch, as we did not test the presence of other birds. We recently demonstrated that after auditory experience with the tutor song, a specific subset of neurons in the NCM shows selective auditory responses to the experienced tutor song (Yanagihara and Yazaki-Sugiyama, 2016). Interestingly, here, we found that the increase response that was observed with tutor presence occurred exclusively in these TUT-selective neurons as well as BOS-selective neurons. Various studies have reported that behavioral states, such as attention or locomotor activity, increase neuronal sensory responsiveness in primary cortical areas without changing spontaneous activity or response tuning (Niell and Stryker, 2010; McGinley et al., 2015). Difference in the amount of response increase with behavioral states between neurons depending on tunings, which is suggested to be an underlying mechanism for enhanced special

resolution with attention (Mineault et al., 2016; Carrasco and Barbot, 2014). In zebra finch, the underlying neuronal mechanism for behavioral state-dependent song learning is still largely unknown. Here we show that specifically to TUT- and BOS- selective neurons, there is an increase in auditory responsiveness with tutor presence. Songbirds offer a good model to investigate neuronal mechanisms of behavioral state control learning with well-identified neuronal circuits and state-dependent learning behavior.

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