Supplementary Information

Title:

Oxytocin receptor antagonism alters context-sensitive song and social brain network activity

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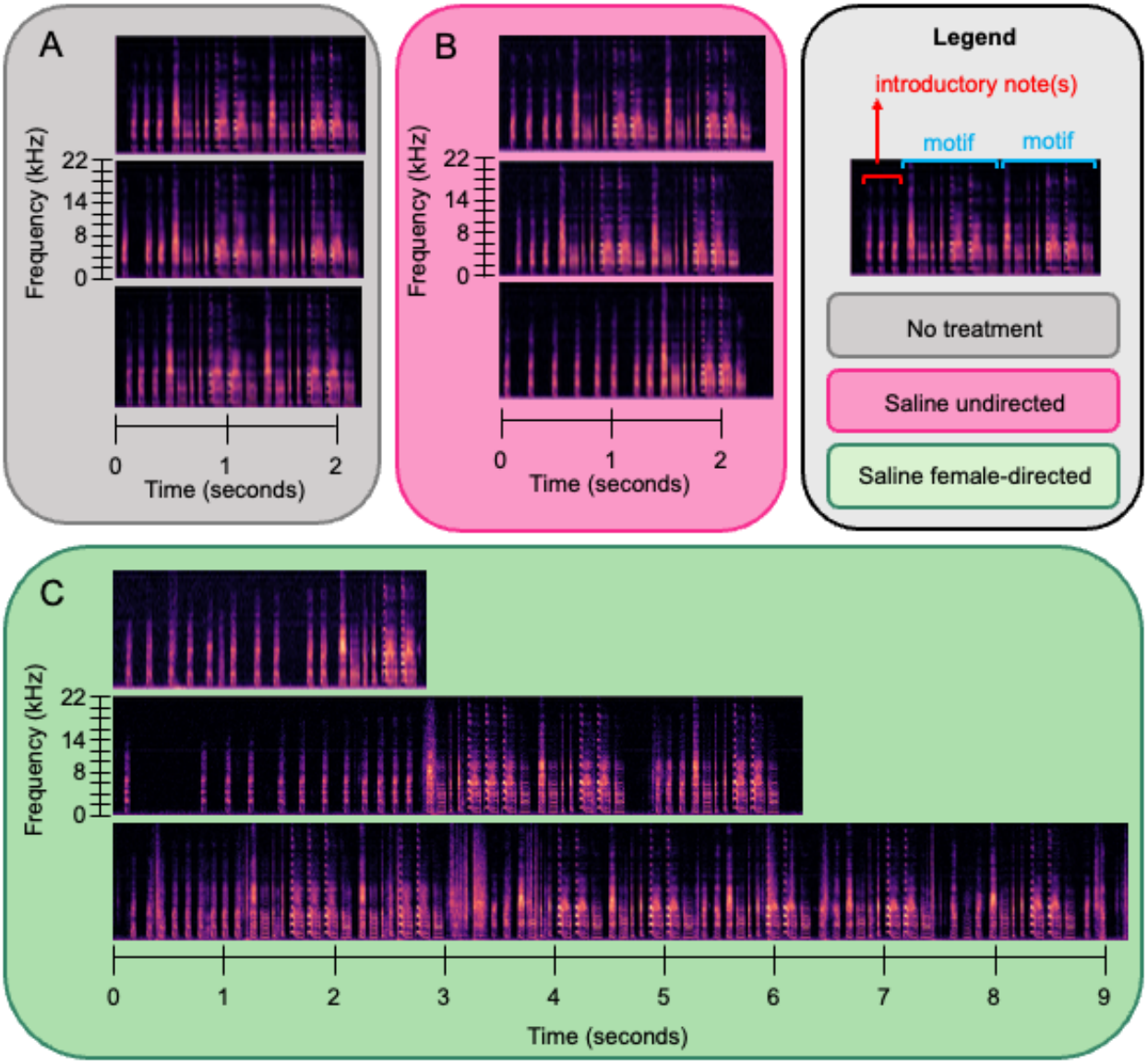
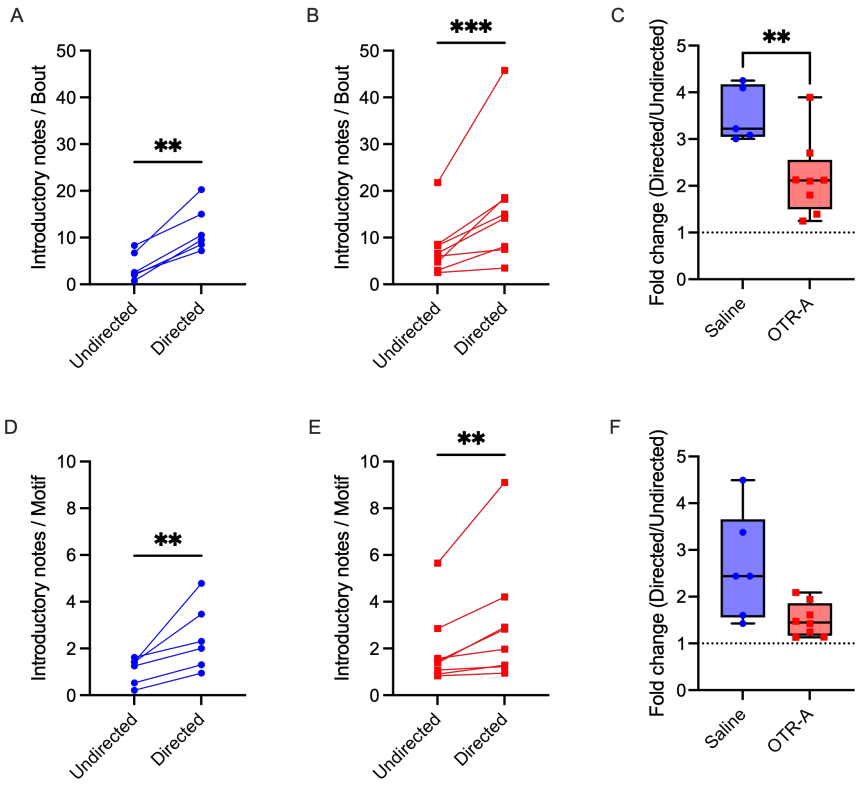
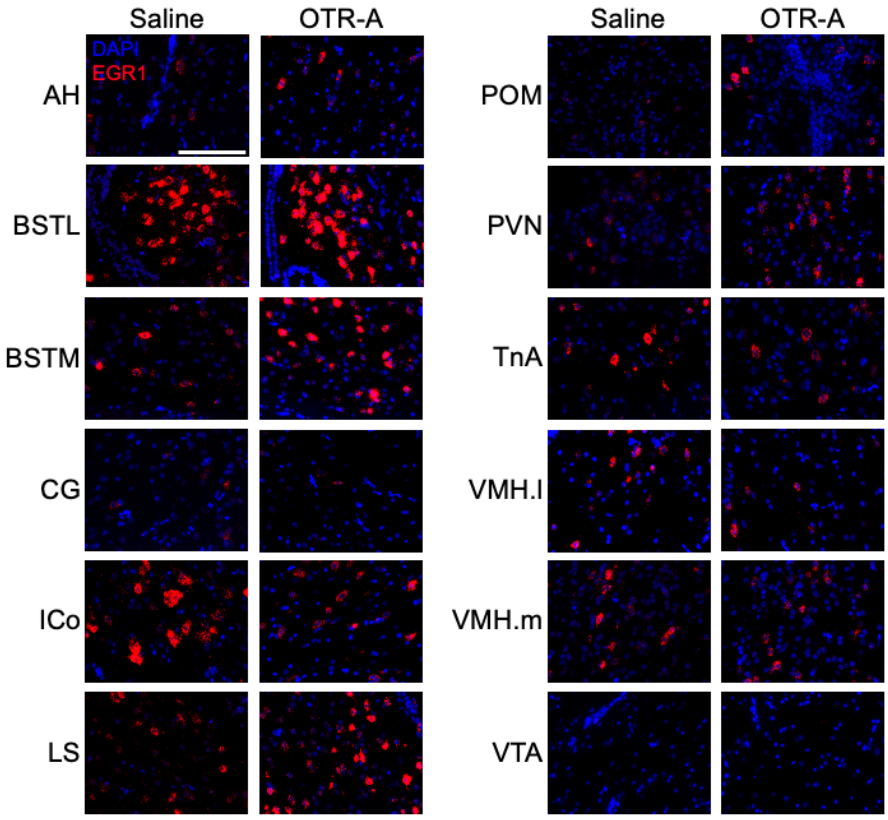


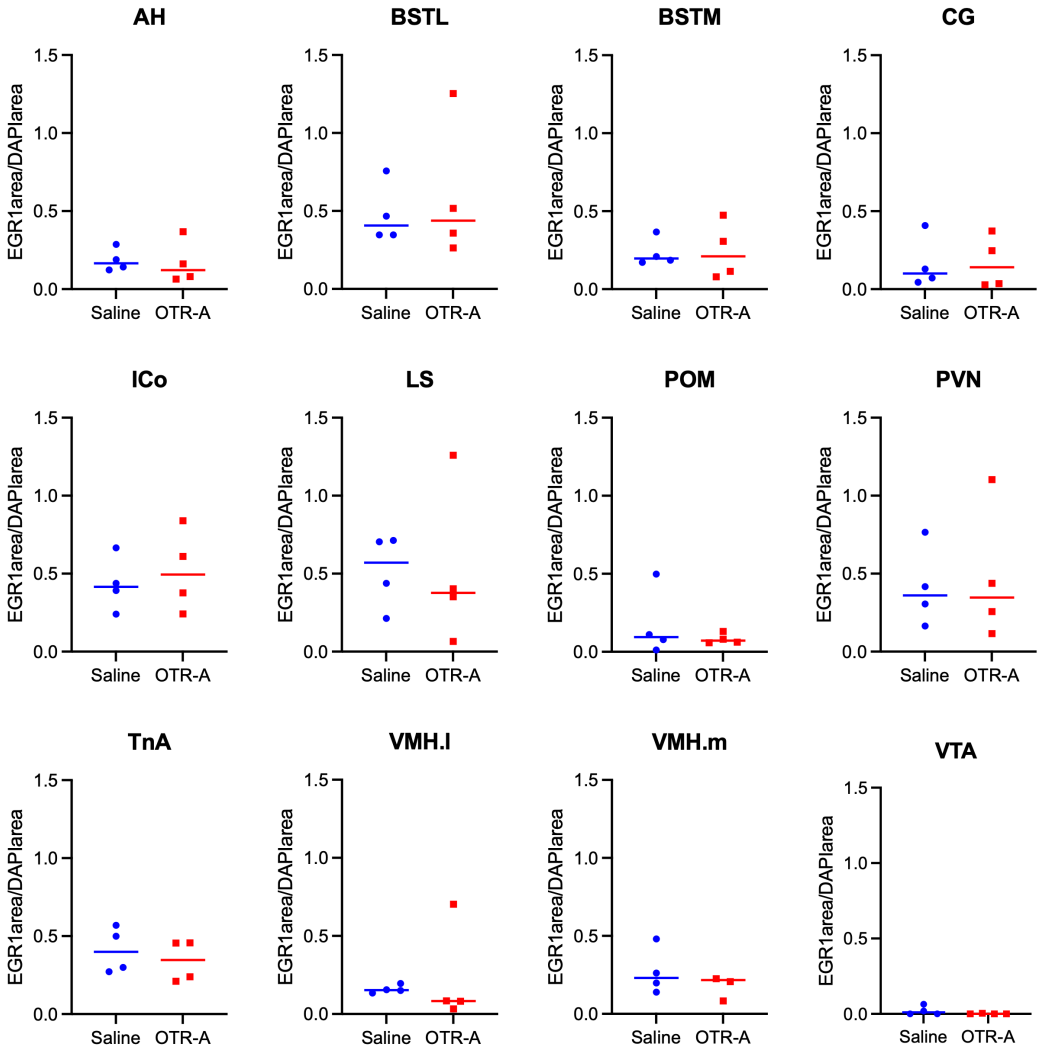
Supplemental Figure 1. Representative treatment-dependent context-sensitive song features. Three randomly selected bouts of song from a single adult male zebra finch in one of three conditions: **(A)** untreated undirected singing, **(B)** saline-treated undirected singing, or **(C)** saline-treated female-directed singing. The structure and location of introductory notes (red) and motifs (blue) in proximity to each other are highlighted in the legend. Each spectrogram shows one bout. All bouts shown were produced during the behavioral observation window. For all spectrograms, the y-axis represents frequency in kilohertz (kHz) and the x-axis represents time in seconds.



Supplemental Figure 2. Introductory notes per bout and per motif increased in female-directed singing contexts, but this effect is diminished by OTR-antagonism. The average number of introductory notes per bout during undirected and female-directed periods of singing are shown for adult male zebra finches treated with either **(A)** saline (n = 6) or **(B)** OTR-antagonism (n = 8). Similarly, the average number of introductory notes per motif within a song bout during undirected and female-directed periods in **(D)** saline-treated (n = 6) or **(E)** OTR-antagonism (n = 7) adult male zebra finches are shown. For all cases, undirected song was produced during an untreated observation period. Ratio paired t-tests were used to determine statistically significant differences for panels A, B, D, and E. **(C & F)** The ratio of introductory notes per bout or per motif for female-directed song compared to undirected song is shown for both saline-treated and OTR-antagonized individuals. Following a robust regression and outlier detection test (ROUT test) to determine outliers, one saline-treated individual was removed from C. Statistical significance of ratios in C and F were calculated with an unpaired two-tailed t-test with a Welch's correction, followed by an F-test to compare variance. Each point denotes data from one individual. * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.



Supplemental Figure 3. Representative fluorescent images for EGR1 expression in all areas of interest in female-directed singing birds. For all social nodes included in this study we present representative images of EGR1 expression at 60X magnification. Images were taken such that the entire image field is the region of interest. All images within each treatment group are from the same individual. The individuals shown for saline and OTR-antagonism groups sang the most female-directed bouts of their respective treatment groups: 20 and 43 bouts, respectively. The scale bar represents 100 μm. Quantification of all reported EGR1 expression is shown in **Supplemental Figure 4**.



Supplemental Figure 4. EGR1 expression in social brain nodes is not dependent on treatment in female-directed singing animals. The ratio between total area of labeled EGR1 and DAPI staining is plotted on the y-axis for female-directed singing adult male zebra finches in saline control (blue circle icons; n = 4) or OTR-antagonism (red square icons; n = 4) conditions for all social nodes of interest to this study. No outliers were detected by a robust regression and outlier detection test (ROUT test) within treatment groups in any node of interest. No statistically significant differences were detected by an unpaired two-way t-test between treatment groups in any node of interest. An F-test was performed to compare variances in saline-treated and OTR-antagonized individuals for all regions.