Supplementary Figure 1. *Syt I* mRNA distribution patterns in zebra finch brain are similar across treatment groups. Representative images from rostral (top) to caudal (bottom) levels. A) Schematic drawings correspond to photomicrographs (B & C) and highlight areas of interest. B) Sections were hybridized to a *Syt I* antisense riboprobe. Side-by-side photomicrographs of film autoradiograms from saline injected (left) or metrazole injected (right) birds reveal similar pallial, striatal and thalamic expression patterns. C) The distribution of *Syt I* in non-singing (left) and singing (right) birds is also similar. Overall, telencephalic *Syt I* levels are high, including within the pallial song nucleus HVC. Levels are low in other song nuclei including LMAN, RA and Area X. (Apparent slight differences in *Syt I* expression between treatment groups are accounted for by slight differences in the plane of sectioning). Scale bar, 1 mm. Abbreviations: A, arcopallium; Cb, cerebellum; H, hyperpallium; HVC, HVC (used as the proper name), LMAN, lateral portion of the magnocellular nucleus of the anterior nidopallium; M, mesopallium; Nc, caudal nidopallium; N, nidopallium; RA, robust nucleus of the archistriatum; St, striatum; X, Area X.

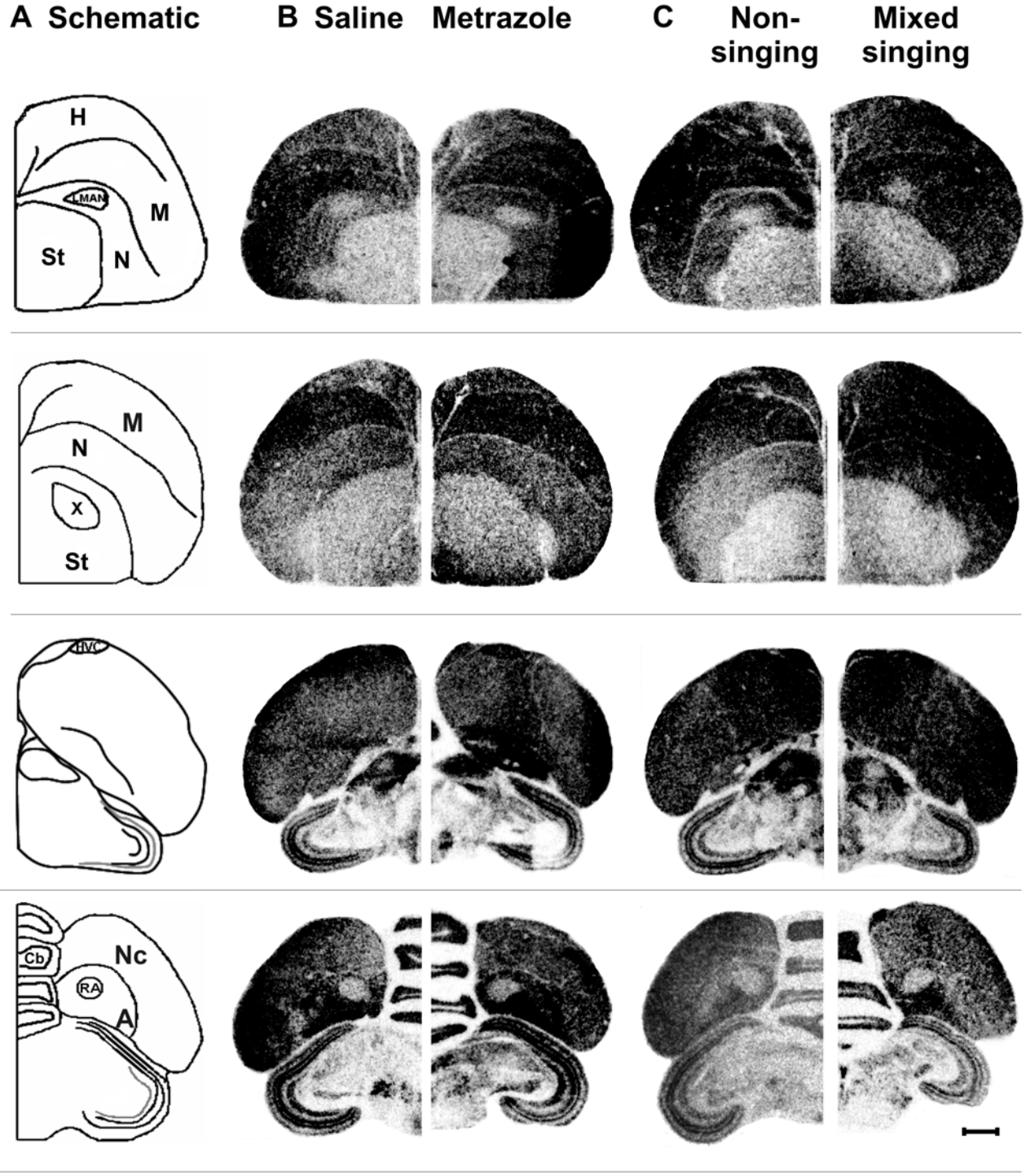
Supplementary Figure 2. *Syt IV* riboprobes are specific for zebra finch *Syt IV*. Photomicrographs of film autoradiograms show exemplar hemi-coronal brain sections (rostral to caudal) hybridized with either the 3' UTR (left) or the full length (right) *Syt IV* riboprobe and reveal highly similar expression patterns. Small differences reflect slightly different planes of section; e.g. the absence (left) or presence (right) of the pretectal nucleus at the level of HVC (arrows). Sense controls show nonspecific signals.

Supplementary Figure 3. *ZENK* mRNA levels demonstrate that metrazole treatment was effective in depolarizing zebra finch brain. Representative photomicrographs show film autoradiograms of hemi-coronal caudal brain sections from birds sacrificed 30 min post-injection. A Nissl-stained section at a plane of section corresponding to that in the other panels reveals neuroanatomical landmarks, including song nucleus RA. Hybridization with the *ZENK* sense probe reveals nonspecific signals. Hybridization with the antisense probe reveals low levels of *ZENK* in a saline injected bird compared with robust *ZENK* induction throughout the telencephalon, cerebellum, and optic tectum in a metrazole injected bird. Note lower levels of induction in RA. Scale bar, 1 mm. Abbreviations: A, arcopallium; Cb, cerebellum; H, hyperpallium; M, mesopallium; Nc, caudal nidopallium; TeO, optic tectum.

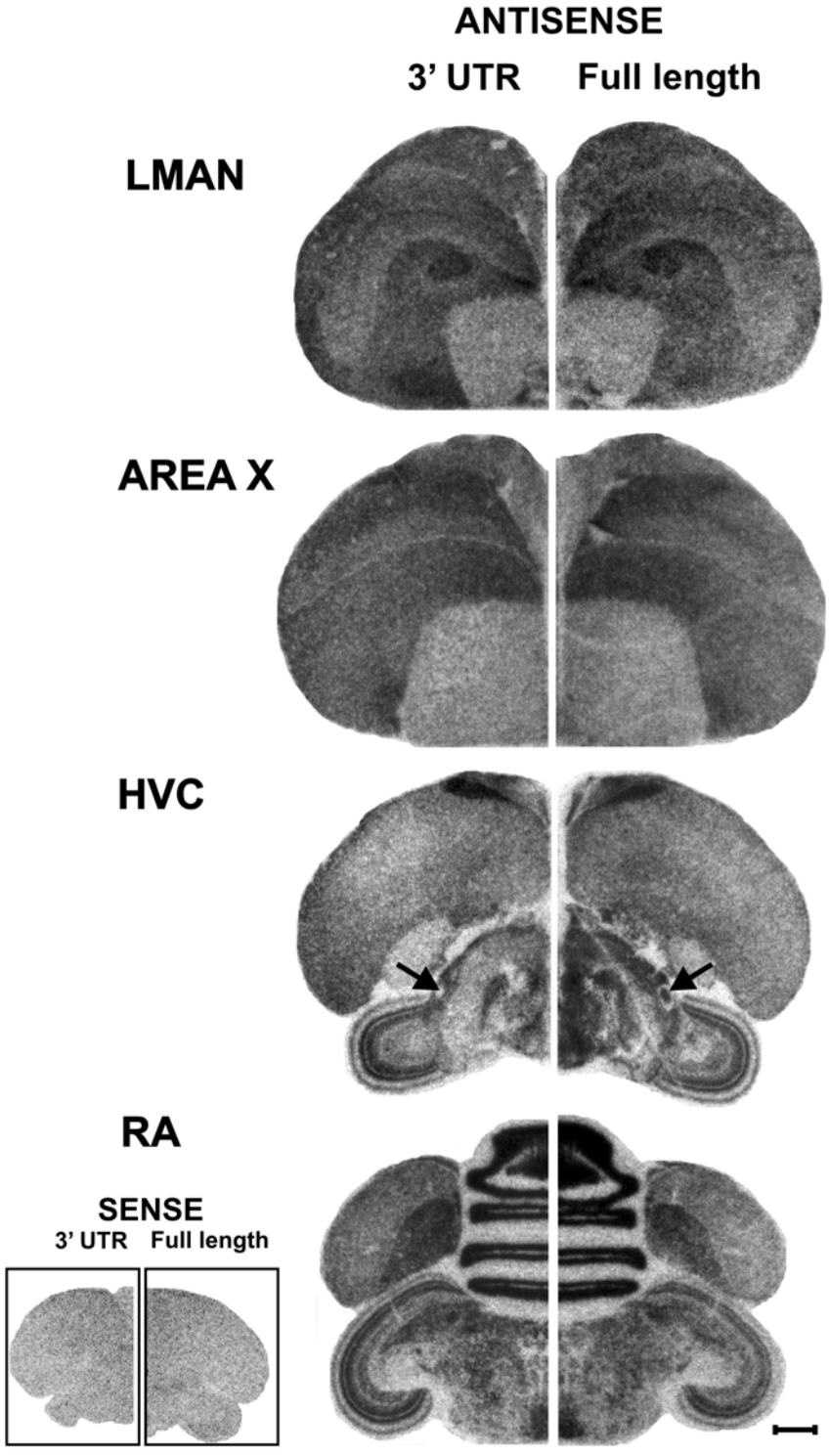
Supplementary Figure 4. *ZENK* expression patterns in Area X indicate that mixed singers sang some undirected songs. Representative photomicrographs of film autoradiograms from hemi-coronal brain sections show *ZENK* signals from a male that sang while housed with a female ('mixed', left), a male whose songs were completely directed towards a female ('directed', middle), and a male that sang while alone ('undirected', right). *ZENK* signals in Area X of the mixed singer resemble those of the undirected singer. Middle and right images are from Teramitsu and White (2006).

Supplementary Table 1. Nidopallial areas selected for normalization do not exhibit changes in *Syt IV* levels across treatment groups. Optical density measurements (OD) from nidopallial areas used for normalization. To minimize variability between *in situ* hybridizations, signals from regions of interest (rostral to caudal: LMAN, Area X, St, HVC, RA, arcopallium and cerebellum) were normalized using an area of the zebra finch nidopallium that did not exhibit changes in gene expression as a function of treatment group. For each region of interest examined (first column), an area of outlying

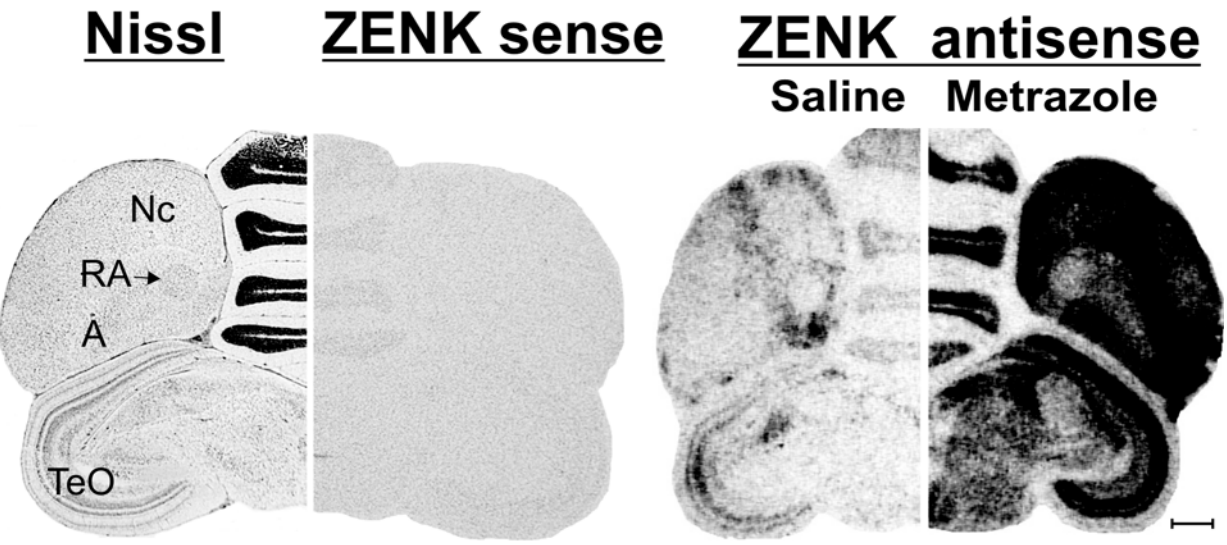
nidopallium used for normalization is indicated by the dotted circles in the corresponding schematic (second column). Average ODs ± SEMs are then indicated, along with the nonsignificant *p*-values. Three to six birds were used to generate each group mean.



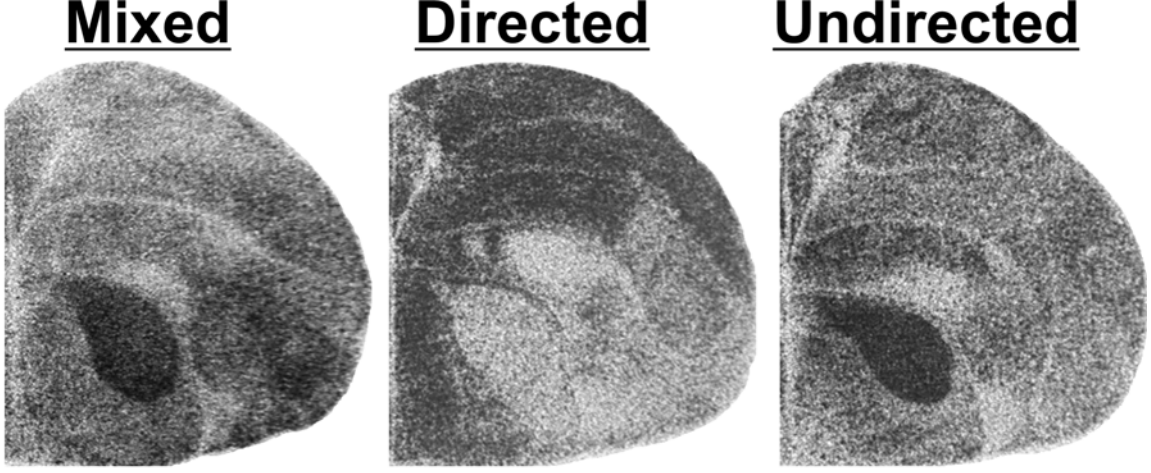
Supplementary Figure 1



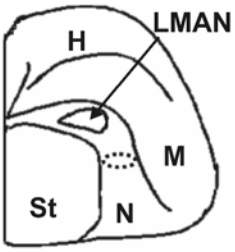
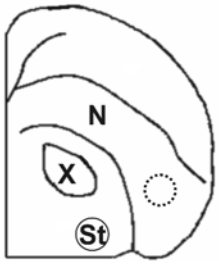
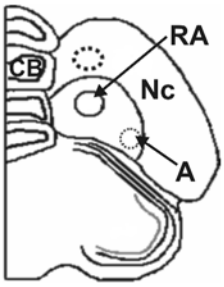
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Regions of Interest	Schematic	Normalization signals from nidopallium: Metrazole	Saline	*p*-value	Mixed singing	Non-singing	*p*-value
LMAN		135 ± 2	133 ± 5	0.92	137 ± 11	125 ± 3	0.67
AREA X & STRIATUM		141 ± 9	137 ± 5	0.86	151 ± 8	145 ± 5	0.67
HVC		116± 9	123	0.33	125 ± 4	133 ± 8	0.70
RA & ARCOPALLIUM		116 ± 7	105 ± 4	0.27	112 ± 2	116 ± 2	0.20
CEREBELLUM		122 ± 10	111 ± 5	0.44	118 ± 3	111 ± 6	0.39

Supplementary Table