Supplemental Information

Cholecystokinin induces crowing in chickens

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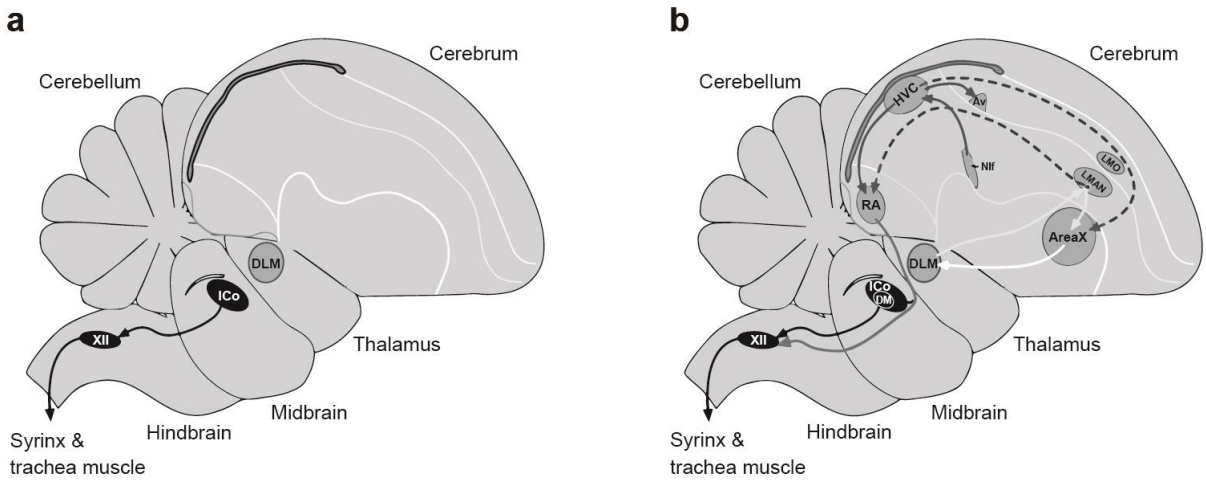
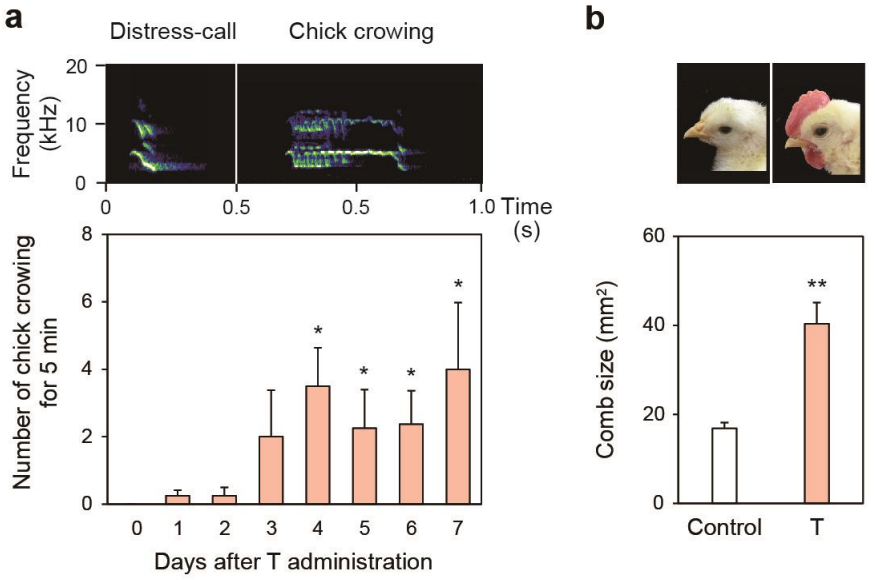
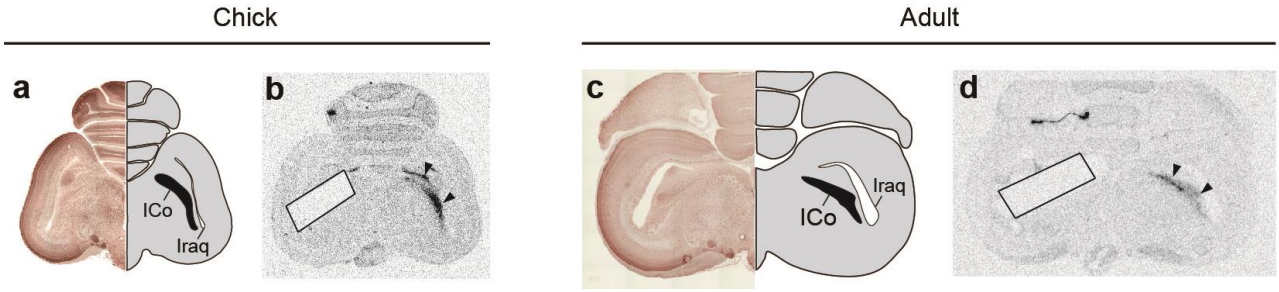


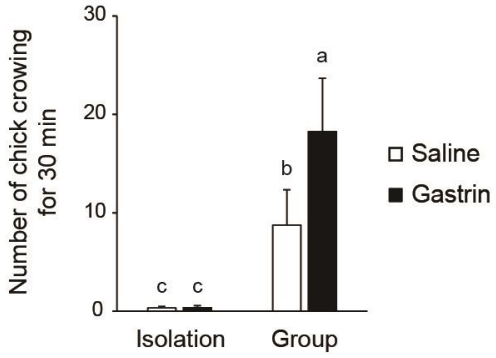
Supplemental Figure S1 | Schematic drawing of brain nucleus and connectivity of the vocal pathway in chicken (non-vocal learner) and zebra finch (vocal learner). (**a**) The forebrain seven song nucleus (**b**) is absent in chickens while the upstream center to produce sounds is located in the midbrain ICo. (**b**) Zebra finches have a complex vocal pathway, including the seven song nucleus. The schematic is adapted from Pfenning et al. (2014). Abbreviations: ICo, nucleus intercollicularis; XII, 12th motor nucleus; NIf, nucleus interfacialis; HVC, high vocal center; Av, avalanche; LMO, lateral oval nucleus of the mesopallium; LMAN, lateral magnocellular nucleus of the anterior nidopallium; RA, robust nucleus of the of arcopallium; DLM, dorsolateral nucleus of the medial thalamus; DM, dorsomedial nucleus of the ICo.



Supplemental Figure S2 | T-administration induces chick crowing. (**a**) Chick crowing of T-administered chicks was observed more frequently 4 d after T administration (H = 15.3, $p < 0.05$, Kruskal-Wallis' test; $p < 0.05$, Shirley-Williams' test, mean ± SEM, n = 8). (**b**) Comb size was bigger in T-administered chicks than control chicks at 7 d after T administration (t = -4.8, $p < 0.01$, t-test, mean + SEM, n = 8). This experiment was independent of the sample collection for RNA-seq (Figure 2).



Supplemental Figure S3 | Validation of ICo punches by *in situ* hybridization. (**a, c**) Grey areas on schematic drawings indicate the ICo. On the opposite side are chromatic images that show the acetylcholine esterase stain. (**b, d**) Black arrowheads on the film indicate expression of the androgen receptor in the ICo. The white areas on the opposite side indicate that the ICo was punched out. Abbreviations: ICo, intercollicular nucleus; Iraq: lateral recess of the aqueduct.



Supplemental Figure S4 | Chick crowing is observed more frequently when the chicks are kept in groups rather than when the chicks are isolated ($F_{1,62}$ = 54.1 [situation, p < 0.01], $F_{1,62}$ = 2.3 [treatment, p > 0.01], $F_{1,62}$ = 7.0 [interaction, p < 0.05], ANOVA; $^{a\text{-}c}p$ < 0.05, Tukey-Kramer's test; mean + SE, n = 8–25). In group situations, 5 chicks were tested at the same time, and the total amount of chick crowing amongst the 5 chicks was counted and used as a single set of data.

Supplemental Table S1 | Probe sequences used in *in situ* hybridization.

Gene Symbol	Probe sequence (antisense sequence)
AR	5’- AAATAAAGGAGTGACCACCCCCAGGTGCTGCACACAGGGCGTTCC -3’
	5’- GGAGATTATGCTCAGCTCTGGACAGGTCCTAGAAGCAGGAGAACC -3’
	5’- GAGCTGCCGCATCCTGACGCATTGGCTGTACATTCTGGATTTGTG -3’
	5’- TGCACAGCCCTGCTGCCTTCGCAGCAGCAAGTAGGTGTATGCAAG -3’
CCK	5’- ATGCTCTGTACCCTGTTCCCTAGGACAGAGAACCTCCCAGTGGAA -3'
	5’- TCCATCCAGCCCATGTAGTCTCTGTCATTTATCCTGTGTGTGGGA -3'
CCKAR	5’- GCAGAAGAGGCAGAGCATTAAGTCACTGACTGCCAGGGACAGCAG -3'
	5’- TTGTGCAGGTAGCACCCGTCTCCATCCTCGTATTTGGCGATGCTG -3'
	5’- TCTTGGCCATCAGGTTGGCAGAAGAGCTGCTGCTTCTCACCCTGT -3'
	5’- GTGTGTGTAAGAGCACTTGGAGAGCGAAGCCCTTGTCGTCTTCCC -3'
CCKBR	5'- GGTTGGGGATGAGGGTGAAGGGCATGCAGCAGAGCGCCAGCATCA -3'
	5'- AATGAAGAAGAGGACGAGGAGCAGCAGGACATACCTGGAAGCGCA -3'
	5'- AGCAGAGGAAGAACATGGCCACGATGACCACCAGCATGCGGATGA -3'
	5'- CAGTGGTGTAGCTGAACTTGGAGAGCGATGCGTTGGCGTTGGCCA -3'
HIST1H2B7L4	5'- TGCACCTGCTTCAGCACCTTGTACACGTAGATCGAGTAGCTCTCC -3'
	5'- GCACCTGCTTCAGCACCTTGTACACGTAGATCGAGTAGCTCTCCT -3'