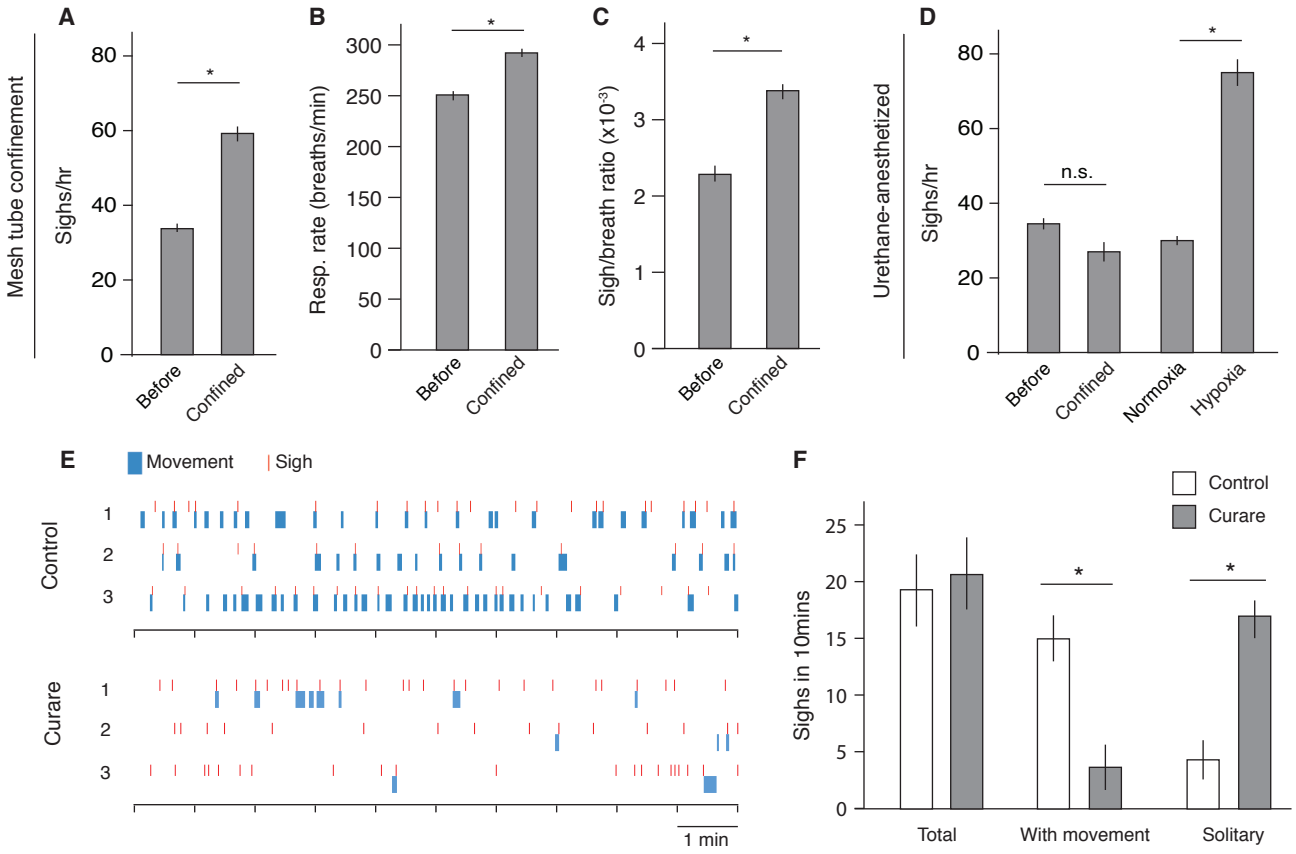


**Figure S1. Effect on breathing of confinement in a larger tube and during anesthesia, Related to Figure 1**
(A to C) Quantification of sigh rate (A), respiratory rate (B) and sigh-to-eupneic-breath ratio (C) before and during confinement of mice (n=8) in a 33 mm diameter metal mesh tube. (Standard assay (Fig. 1B to 1D) uses 27 mm diameter plastic tube.) (D) Quantification of sigh rate in mice (n=4) anesthetized with urethane in standard confinement assay or under hypoxia (10% O2). Note anesthesia eliminates confinement-induced (emotional) sighing but not hypoxia-induced (physiological) sighing. (E) Activity traces during the confinement assay showing bouts of movement (blue bars) and sighs (red ticks) in control mice (vehicle-injected; n=3 mice, top three traces) and mice treated with curare to inhibit skeletal muscle movement (tubocurarine-injected; n=3 mice, bottom three traces). (F) Quantification of E showing total sighs during the 10 minute assay, and sighs stratified by those that occurred with movement ("with movement") or without ("solitary"). Open bars, control mice; grey bars, curare-treated mice. Data shown are mean +/- SEM. *, $p<0.05$ (paired t-test); n.s., not significant.

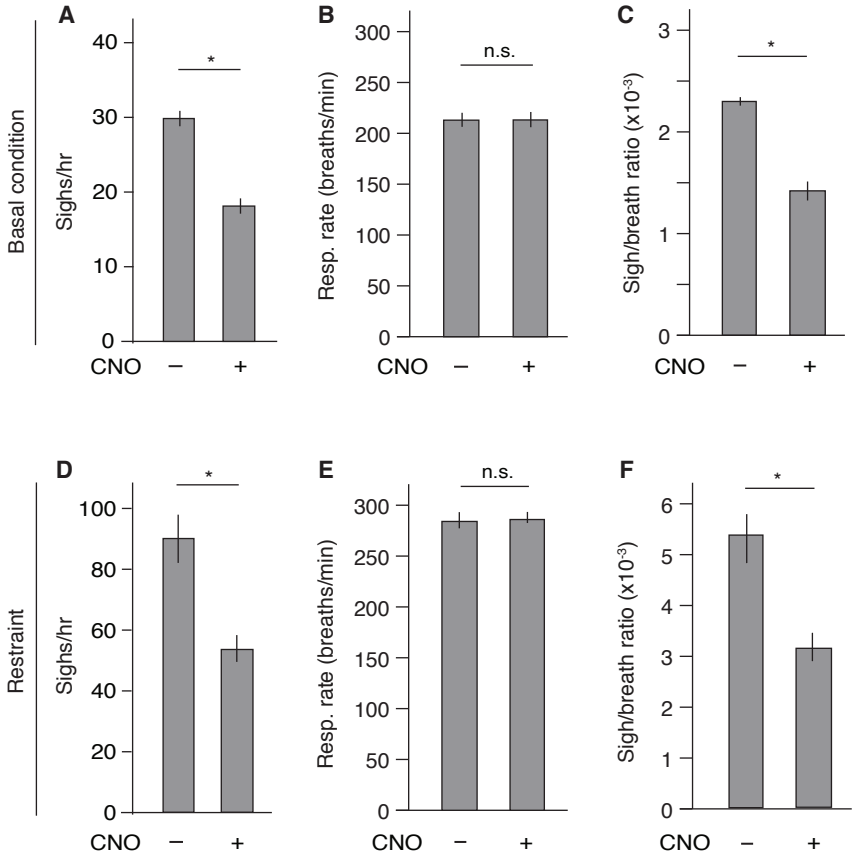


**Figure S2. Effect on breathing of RTN Nmb neuron silencing during tube confinement, Related to Figure 1**
(A to F) Sigh rate (A and D), respiratory rate (B and E), and sigh-to-eupnic-breath ratio (C and F) under basal conditions (A to C) or during tube confinement (D to F) of mice (n=6) expressing hM4Di in RTN Nmb neurons that were treated with vehicle alone (-) or with CNO to silence Nmb neurons as shown in Fig. 1D. Note difference in scale between panels A to C and corresponding panels in D to F. The last two bars of Fig. 1H are reproduced here (panel D) for comparison. Data shown are mean +/- SEM. *, $p<0.05$ by paired t-test.

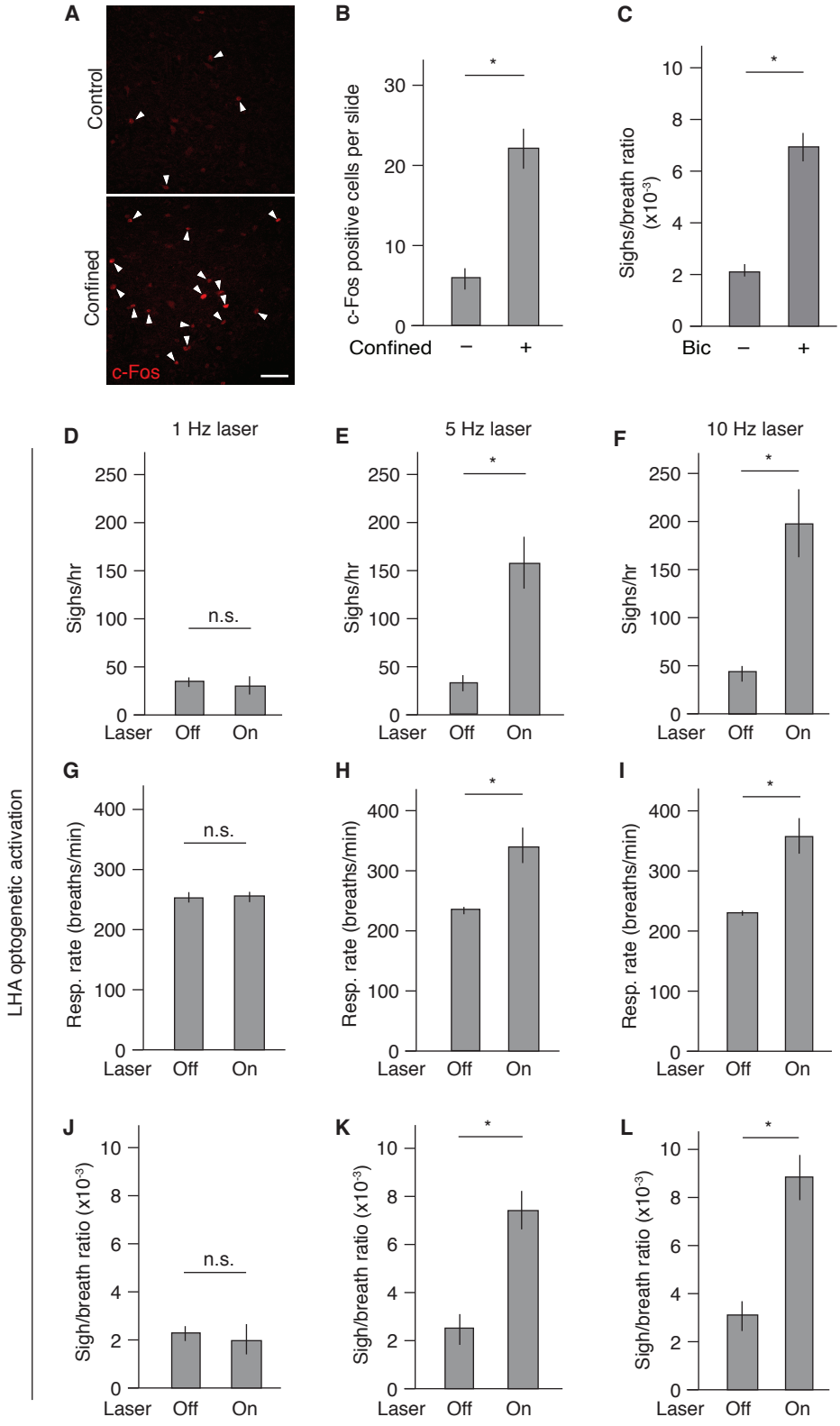


**Figure S3. LHA neurons are activated during confinement and their optogenetic activation with 5 or 10 Hz laser induces sighing and tachypnea, Related to Figure 3**

(A) Mouse LHA brain slices immunostained with c-Fos to show active neurons (arrowheads) under control and tube confinement conditions. Scale bar, 50 um. (B) Quantification of c-Fos positive neurons in control and tube confinement conditions as in A. (C) Quantification of sigh-to-eupnic-breath ratio of anesthetized wild-type C57BL/6 mice (n=6) before and after stereotactic bicuculline (Bic) injection to disinhibit LHA (see Fig. 1c,d). (D to L), Sigh rate (D to F), respiratory rate (G to I), and sigh-to-eupnic-breath ratio (J to L) of wild-type mice (n=6) before (laser off) and during (laser on) photoactivation of LHA neurons with laser power at 1 (D, G, J), 5 (E, H, K) or 10 Hz (F, I, L). Note respiratory effects of 5 or 10 Hz but not 1 Hz laser photoactivation. Data from Fig. 3E and F are reproduced here (panel E) for comparison. Data shown are mean +/- SEM. *, $p<0.05$ by paired t-test.

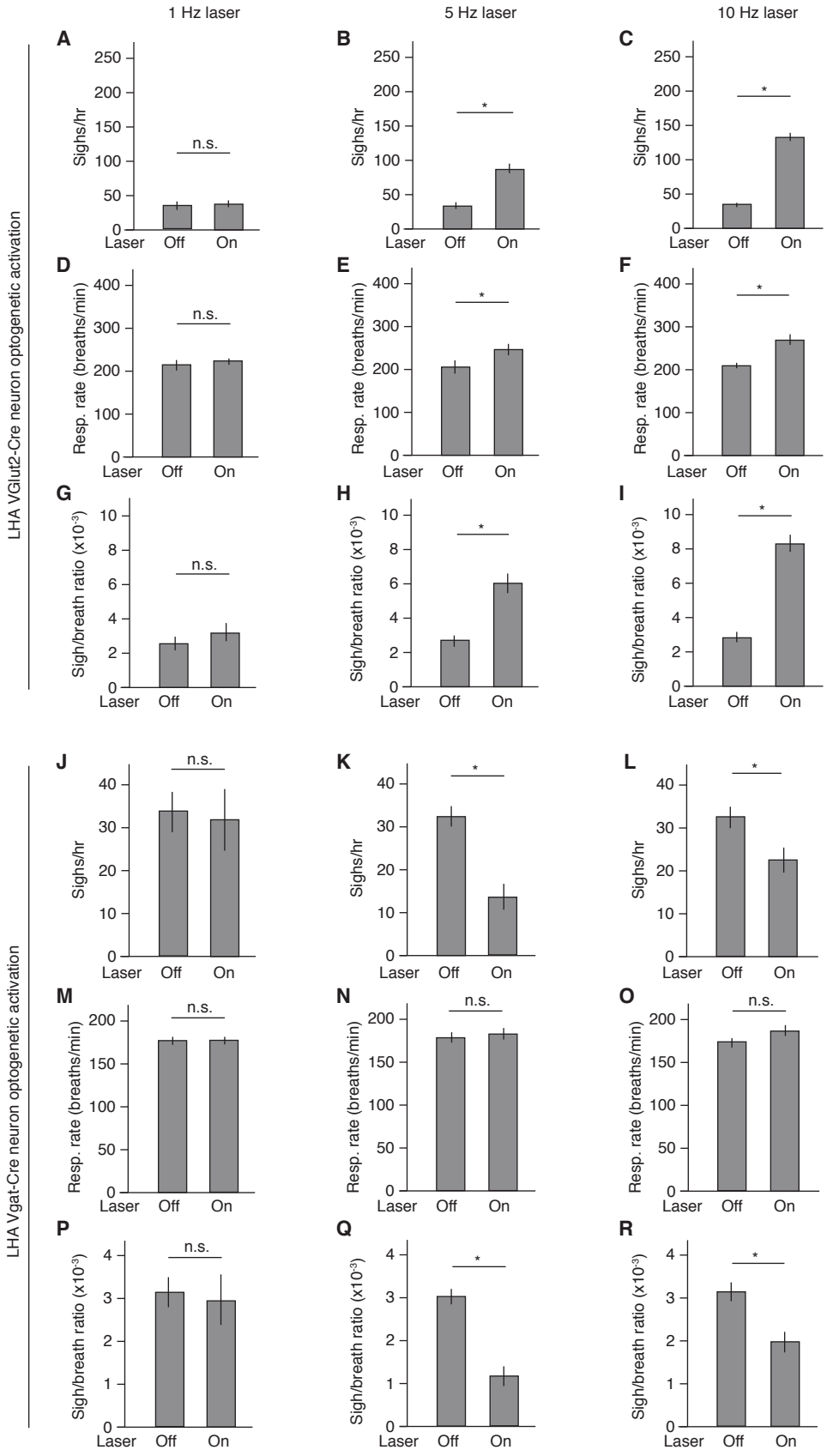


**Figure S4. Effect of optogenetic activation of LHA glutamatergic and GABAergic subpopulations on breathing, Related to Figure 3**

(A to I) Sigh rate (A to C), respiratory rate (D to F), and sigh-to-eupnic-breath ratio (G to I) of anesthetized adult Vglut2-IRES-Cre knock-in mice (n=6) injected into LHA with AAV-DIO-ChR2 then photoactivated 2 weeks with blue laser at 1 (A, D, J), 5 (B, E, H) or 10 Hz (C, F, I) to optogenetically activate LHA Vglut2+ (glutamatergic) neurons and analyzed by spirometry as in Fig. 4A. Data from Fig. 4A are reproduced here (panel C) for comparison. (J to R) Sigh rate (J to L), respiratory rate (M to O), and sigh-to-eupnic-breath ratio (P to R) of anesthetized adult Vgat-IRES-Cre mice (n=6) treated as above (and in Fig. 4B) to optogenetically activate LHA Vgat+ (GABAergic) neurons. Data from Fig. 4B are reproduced here (panel K) for comparison. Data shown are mean +/- SEM. *, $p<0.05$ by paired t-test.

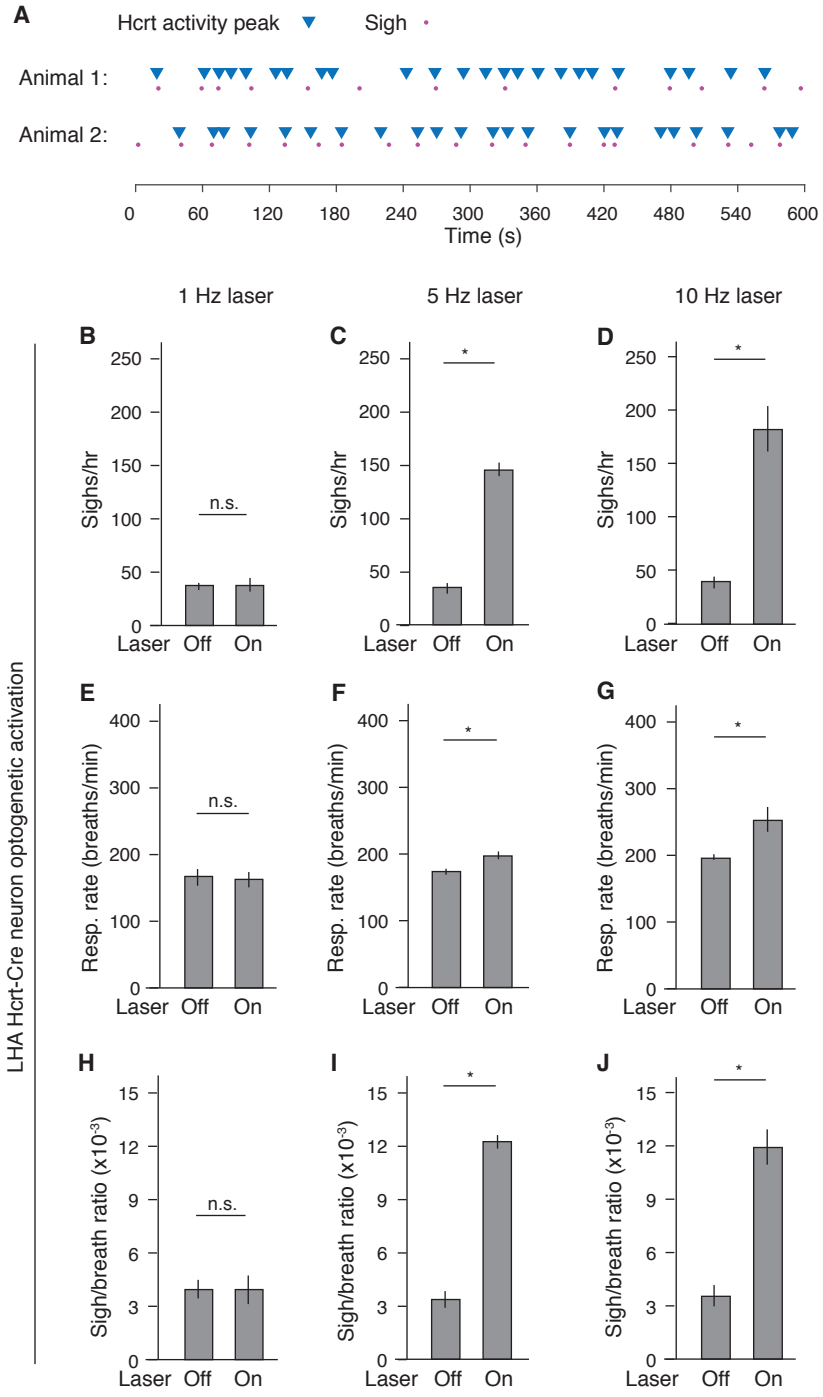


**Figure S5. Effect of optogenetic activation of LHA Hcrt+ neurons on breathing, Related to Figure 4**
(A) Timing of Hcrt neuron activity peaks (triangle) and the sighs (red dot) during tube confinement by simultaneous recording of Hcrt neurons (as shown in (B to J) Sigh rate (B to D), respiratory rate (E to G), and sigh-to-eupnic-breath ratio (H to J) of anesthetized adult Hcrt-IRES-Cre knock-in mice (n=6) injected into LHA with AAV-DIO-ChR2 then photoactivated 2 weeks with blue laser at 1 (B, E, H), 5 (C, F, I) or 10 Hz (D, G, J,) to optogenetically activate LHA Hcrt+ neurons and analyzed by spirometry as in Fig. 4C. Data from Fig. 4C are reproduced here (panel C) for comparison. Data shown are mean +/- SEM. *, $p<0.05$ by paired t-test.

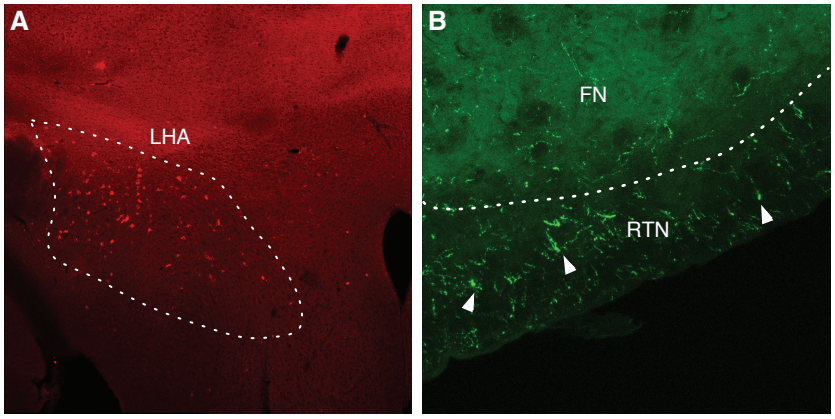


**Figure S6. LHA neurons project to RTN region, Related to Figure 5**
(A) LHA brain slice of adult mouse injected in RTN with retrobeads fluorescent retrograde tracer (red) and analyzed 5 days later. Note the retrograde-labeled neurons in LHA (dashed outline) but not the surrounding brain regions. (B) RTN brain slice of adult mouse injected into LHA with a YFP-expressing AAV vector (AAV-hSyn1-eYFP) and analyzed two weeks later. Note more YFP-labeled LHA neuronal processes (arrowheads) in RTN than in neighboring facial nucleus (FN).

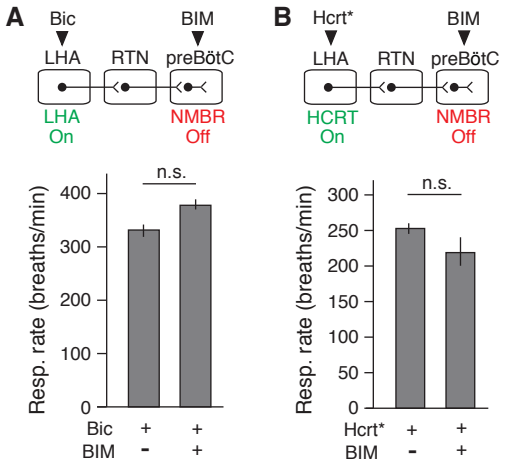


**Figure S7. Effect of NMBR antagonism on respiratory rate during activation of LHA and Hcrt neurons, Related to Figure 6**

(A) Respiratory rate after bicuculline injection to de-repress LHA of anesthetized mice with NMBR antagonist BIM23042 (BIM, +; n=7) or vehicle alone (-; n=6) injected in preBötC as in Fig. 5J. (B) Respiratory rate during optogenetic activation of LHA Hcrt-Cre neurons (Hcrt*) of anesthetized mice with BIM23042 (BIM, +; n=6) or vehicle alone (-; n=6) injected into preBötC as in Fig. 5K. Data shown are mean +/-SEM; *, $p<0.05$ by unpaired t-test.