

Arcuate Neuropeptide Y decreases sympathetic nerve activity via multiple neuropathways.

Supplement

by

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Running title: Arcuate NPY decreases SNA via PVN and DMH

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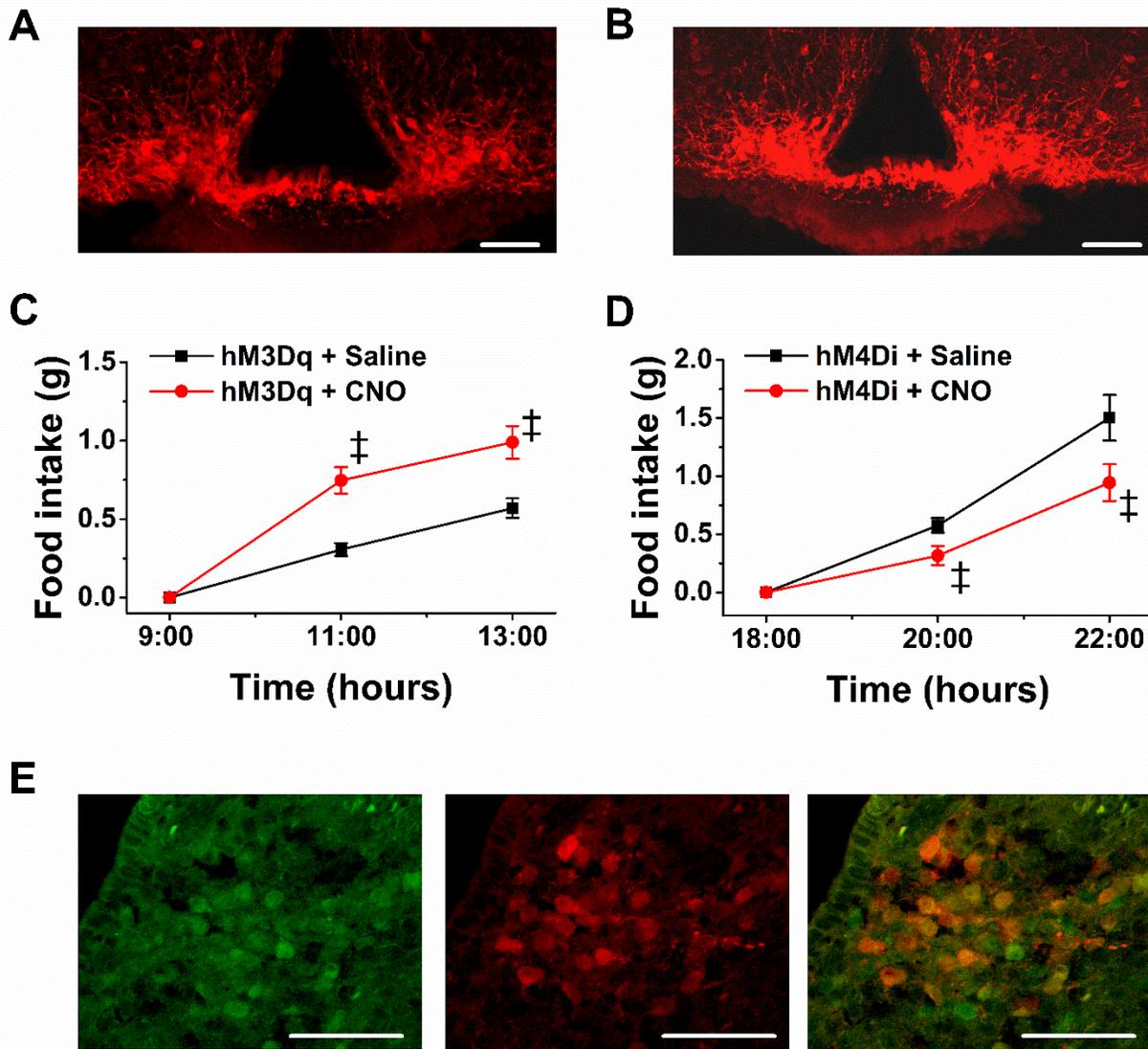


Figure S1. hM3Dq or hM4Di is selectively expressed in ArcN NPY neurons. (A). ArcN hM3Dq mCherry expression. (B) ArcN hM4Di mCherry expression. (C) Food intake is stimulated by hM3Dq activation of ArcN NPY/AgRP neurons in mice tested during the light (sleep) cycle. (D). Food intake is inhibited by hM4Di inhibition of ArcN NPY/AgRP neurons in mice tested at lights off (beginning of wake cycle). (E). In sections from 3 mice, all hM3Dq mCherry expressing ArcN neurons (middle) also exhibit NPY-GFP (left). Colocalization is indicated by the yellow-orange color in the merged image (right). The white scale bars equal 100 μ m.

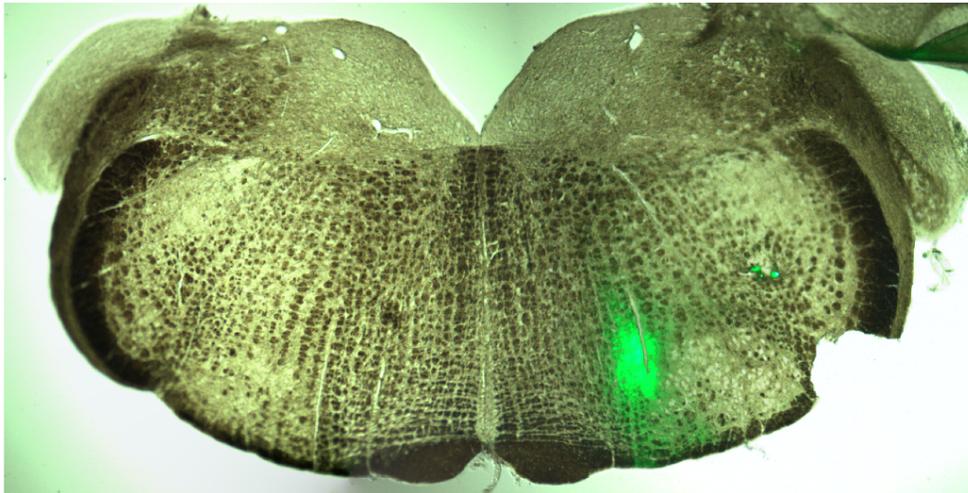


Figure S2. RVLm CtB injection sites in 3 *AgRP-ires-Cre* mice that also received the AAV8.2-hEF1alpha-DIO-Synaptophysin-mCherry-WPRE vector into the ArcN.

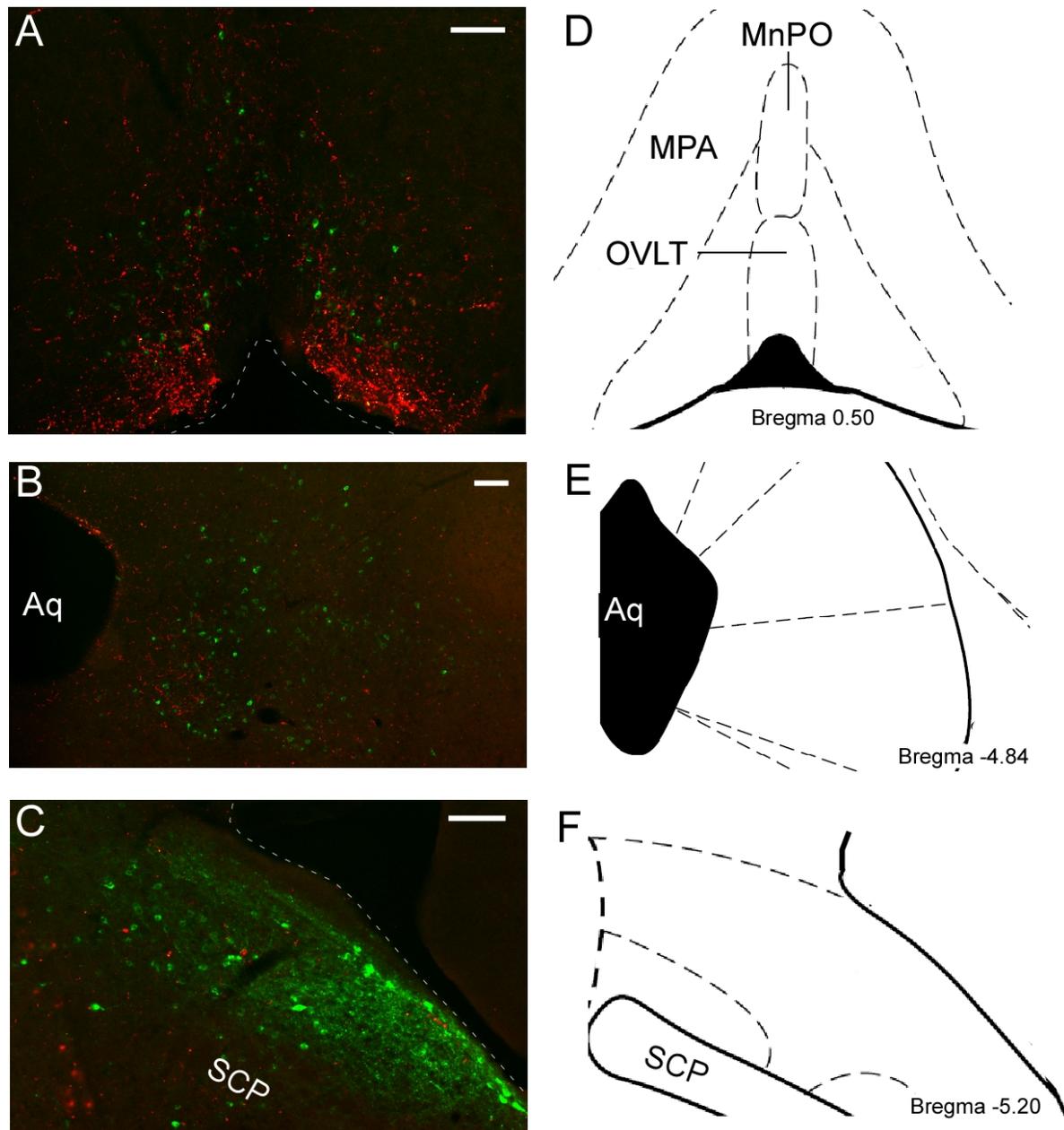


Figure S3. In the mouse, the preoptic area (POA), the ventrolateral periaqueductal gray (vIPAG) and the lateral parabrachial nucleus (LPB) project to the RVLM, and these regions also receive ArcN NPY/AgRP inputs. CtB-immunoreactive (ir) neurons (green) and Ds-red-ir fibers and terminals in the POA (A), vIPAG (B) and LPB (C) following injection of CtB in the RVLM (injection sites illustrated in Figure S2) and cre-dependent mCherry expression in NPY/AgRP neurons of the ArcN. The areas depicted in panels A, B, and C are indicated by the red squares in panels D, E, and F, respectively. Aq, aqueduct; SCP, superior cerebellar peduncle. The white scale bars are equal to 100 μm.

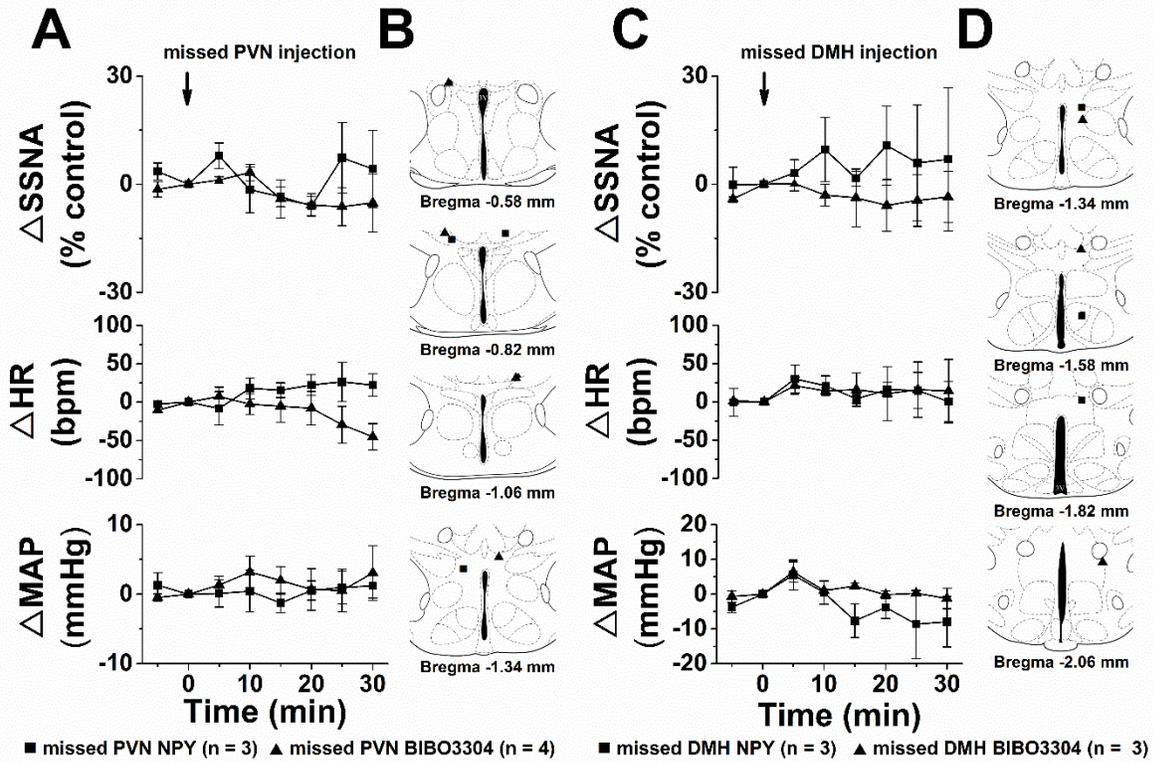


Figure S4. Nanoinjections of NPY or BIBO3304 that miss the PVN or DMH fail to significantly alter SSNA, HR, and MAP. (A). Grouped data showing the changes in SSNA, HR, and MAP from baseline levels of 96 ± 6 mmHg and 454 ± 26 bpm ($n=7$) in animals in which NPY or BIBO3304 nanoinjections missed the PVN. (B). Histological locations of missed PVN injections. (C). Grouped data showing the changes in SSNA, HR, and MAP from baseline levels of 87 ± 7 mmHg and 466 ± 32 bpm ($n=6$) in animals in which NPY or BIBO3304 nanoinjections missed the DMH. (D). Histological locations of missed DMH injections. Histological maps adapted from (Paxinos G. and Franklin, 2001).

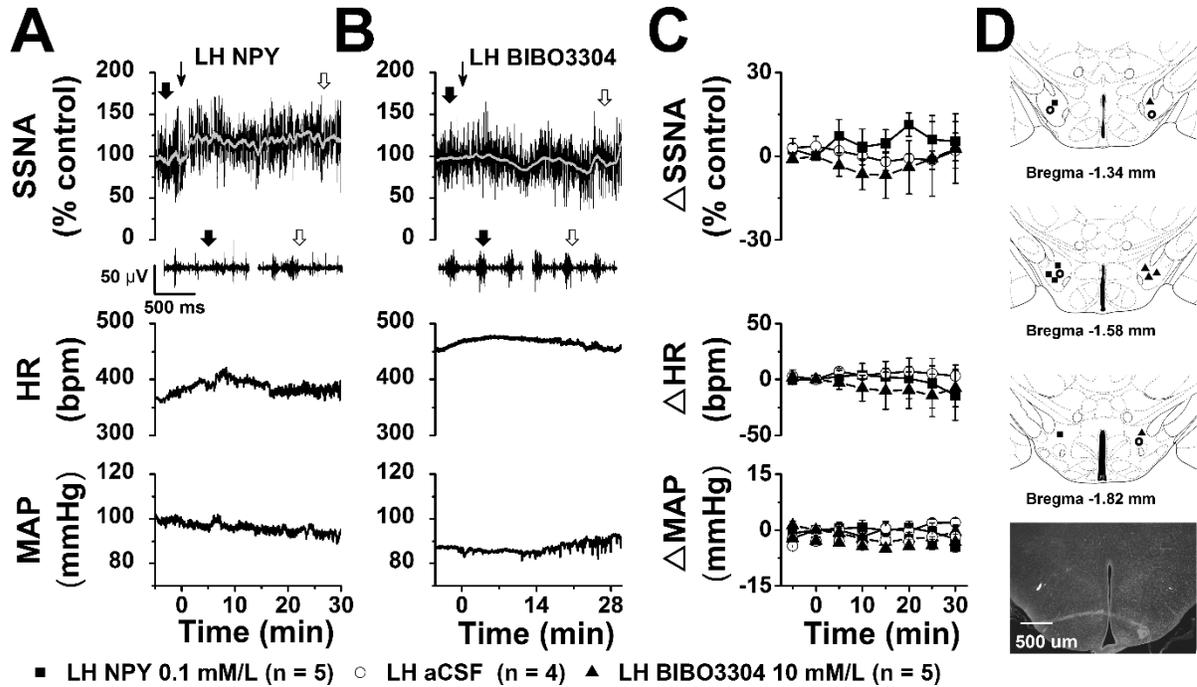


Figure S5. Bilateral nanoinjections of NPY or BIBO3304 into the LH of WT mice have no significant effects on SSNA, HR, and MAP. (A) Representative experiment showing that injections (at thin arrow) of NPY into the LH have variable effects on raw SSNA, HR and MAP. (B). Representative experiment showing that injections (at thin arrow) of BIBO3304 into the LH Have variable effects on raw SSNA, HR and MAP. (C). Grouped data showing that overall LH NPY, BIBO3304, or aCSF injections had no effects on SSNA, HR and MAP. Baseline values were 79 ± 3 mmHg and 455 ± 23 bpm ($n=14$). (D). Histological maps, adapted from (Paxinos G. and Franklin, 2001), and representative sections illustrating LH injection sites.

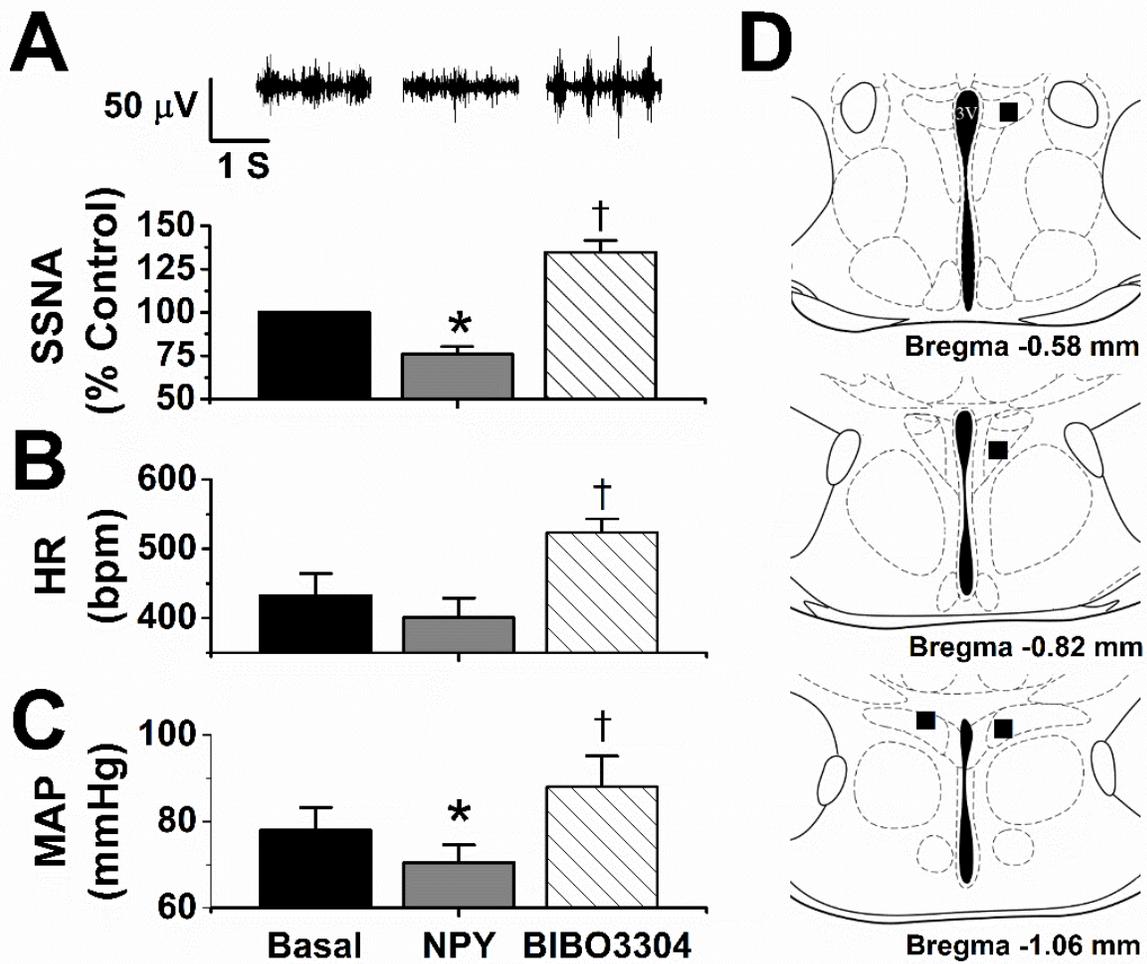


Figure S6. PVN BIBO3304 reverses the effects of PVN NPY. PVN NPY decreases SSNA (A), HR (B), and MAP (C), and subsequent PVN BIBO3304 increases these variables above baseline, similarly to the effect of PVN BIBO3304 in untreated animals (Figure 3C). D. Injection sites. *: $P < 0.05$, compared to time 0; †: $P < 0.05$ compared to previous value within group.

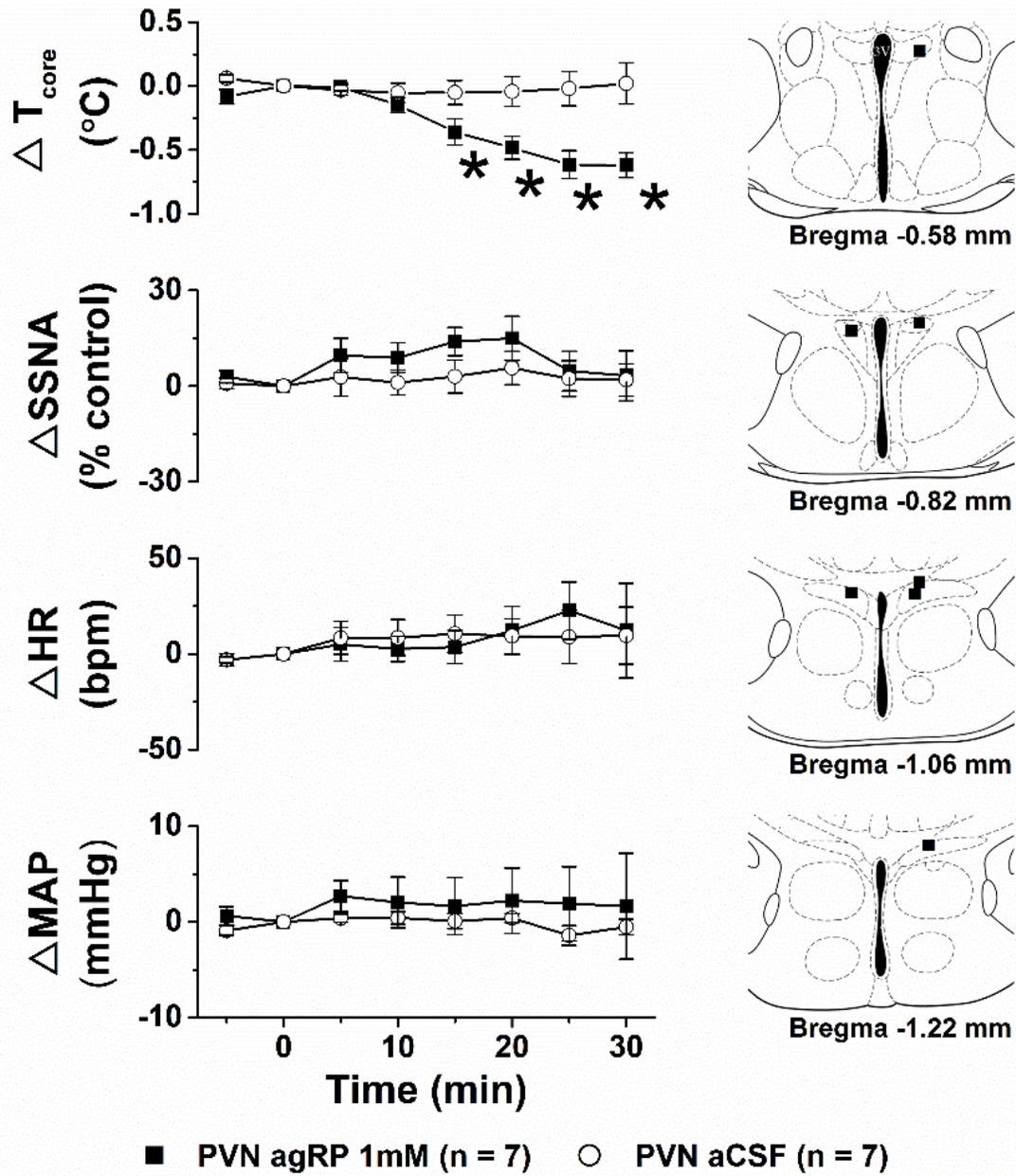


Figure S7. PVN nanoinjections of AgRP decreased body temperature (ΔT_{core}), but had no effect on SSNA, HR, and MAP (left panel). Baseline values were 88 ± 6 mmHg and 424 ± 19 bpm ($n=7$). Right panel: Injection sites. *: $P < 0.05$, compared to time 0.

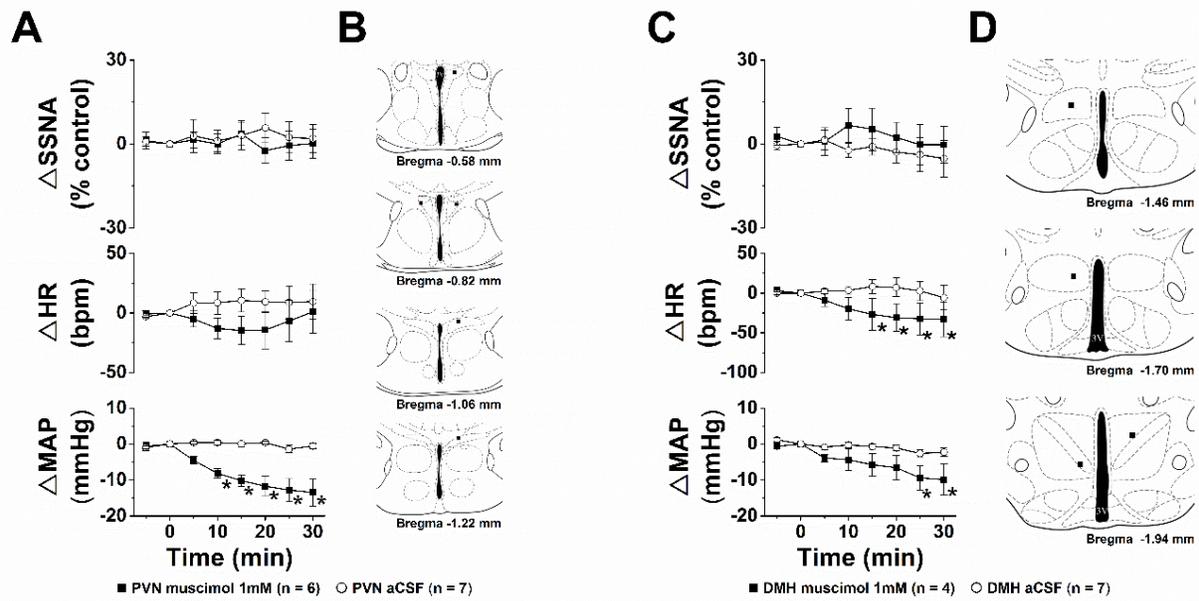


Figure S8. PVN or DMH nanoinjections of muscimol have minimal effects on SSNA, MAP and HR. (A). Grouped data showing that PVN injections of muscimol decrease MAP, but have no effect on SSNA and HR (C). Grouped data showing that DMH injections of muscimol slightly decrease MAP and HR, but have no effect on SSNA. (B, D). Histological maps of PVN and DMH injections sites. Maps adapted from (Paxinos G. and Franklin, 2001). *: $P < 0.05$, compared to time 0.

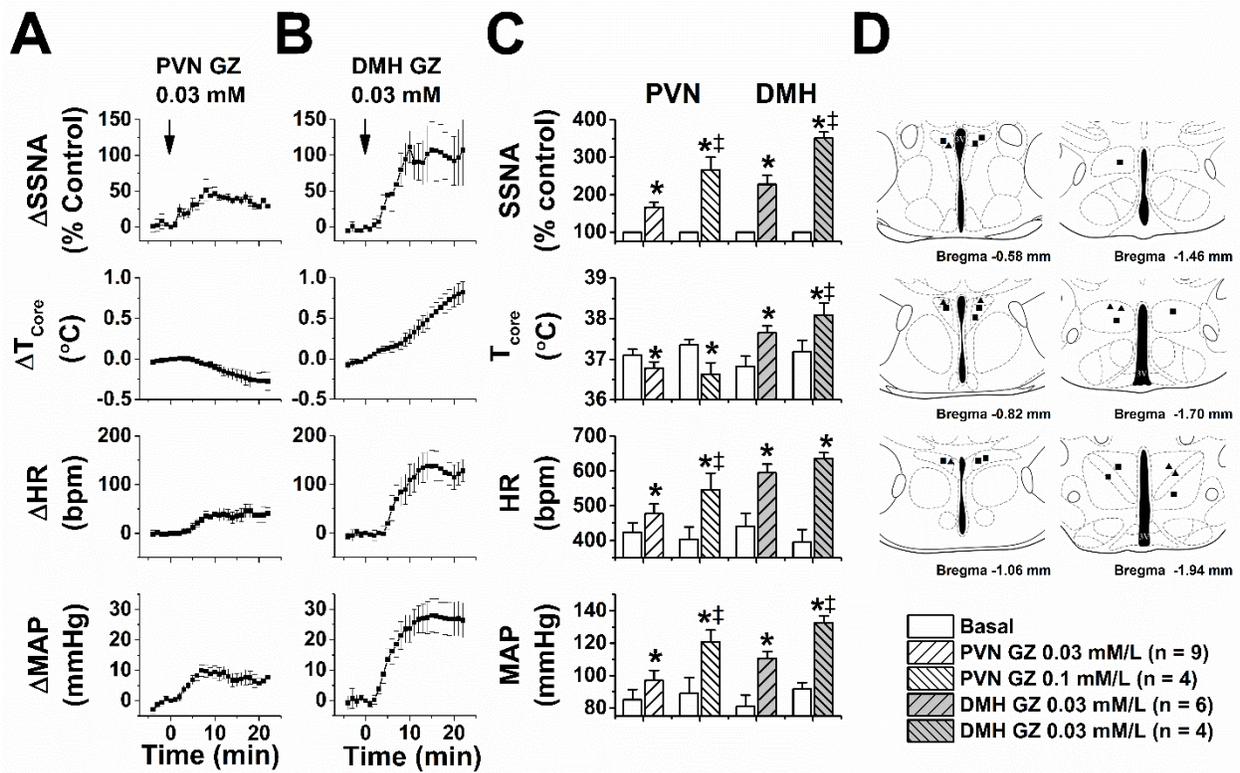


Figure S9. Nanoinjections of gabazine into the PVN and DMH dose dependently increase SSNA, HR, and MAP. (A, B). Grouped data showing the time course of effects of PVN or DMH injections of the lower dose of the GABA_A receptor antagonist, gabazine, to increase SSNA, HR, and MAP. Interestingly, while DMH gabazine increased body temperature (ΔT_{core}), PVN gabazine decreased T_{core} . (C). Nanoinjections of gabazine into the PVN and DMH dose dependently increased SSNA. PVN gabazine also dose dependently increased MAP and HR, while only the effect of DMH gabazine on MAP was dose-dependent (i.e. the effect on HR was maximal at the lower dose). Again, while PVN gabazine decreased ΔT_{core} , DMH gabazine dose-dependently increased it. (D) Histological maps adapted from (Paxinos G. and Franklin, 2001) illustrating injection sites. *: $P < 0.05$, compared to time 0; †: $P < 0.05$ compared to response to lower dose.

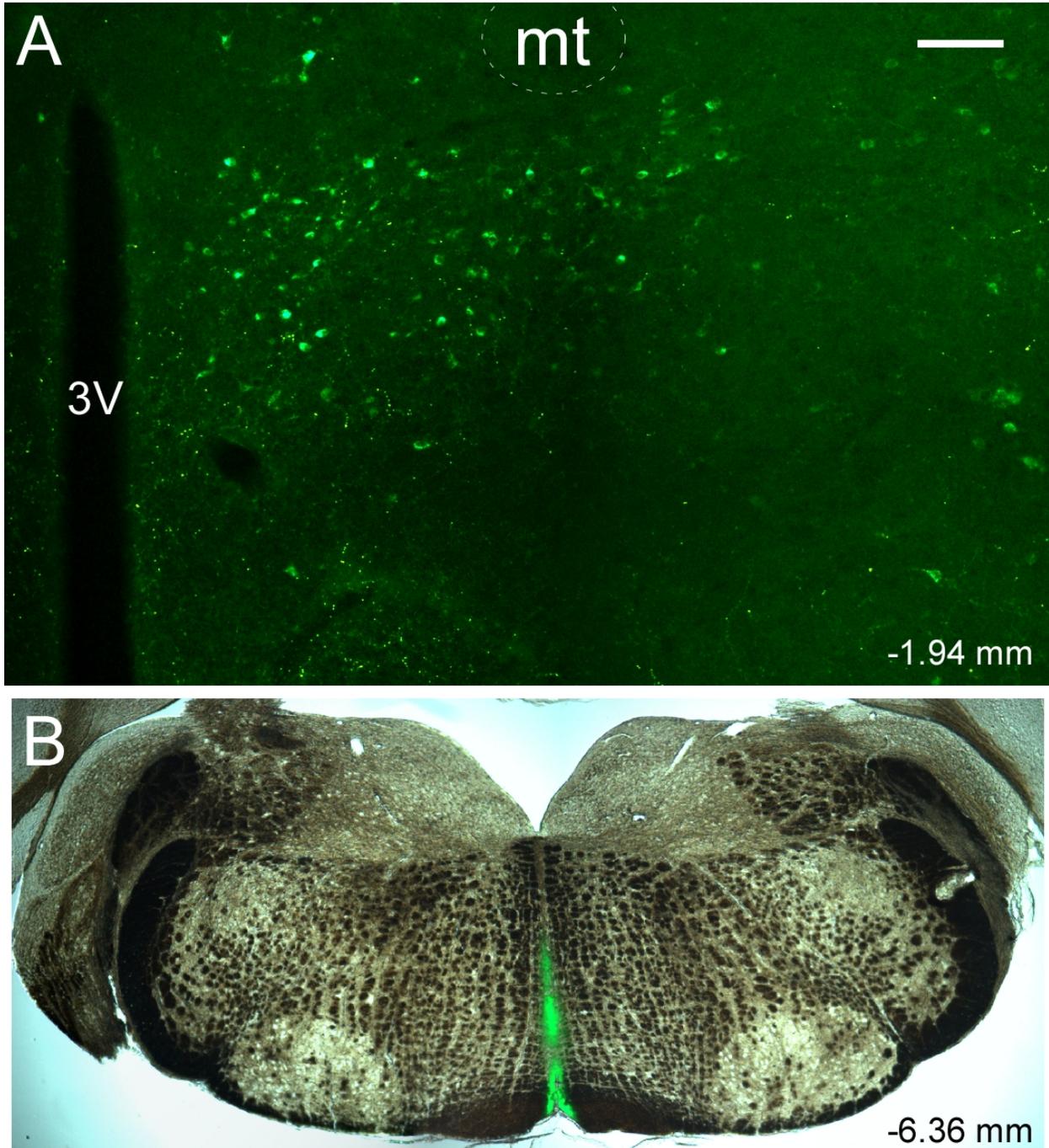


Figure S10. DMH neurons project to the Raphe Pallidus (RPa) of the mouse. (A) Photomicrograph of a partial coronal section (centered on the right hemisphere) of the hypothalamus illustrating CtB-containing neurons (green) that were retrogradely-labeled following CtB injection (green) in the rostral raphe pallidus area (rRPa) (shown in B). Numbers represent the approximate distance from bregma of the representative coronal sections based on the atlas of (Paxinos G. and Franklin, 2001). 3V, third ventricle; mt, mammillary tract. The white scale bar in A equals 100 μ m.

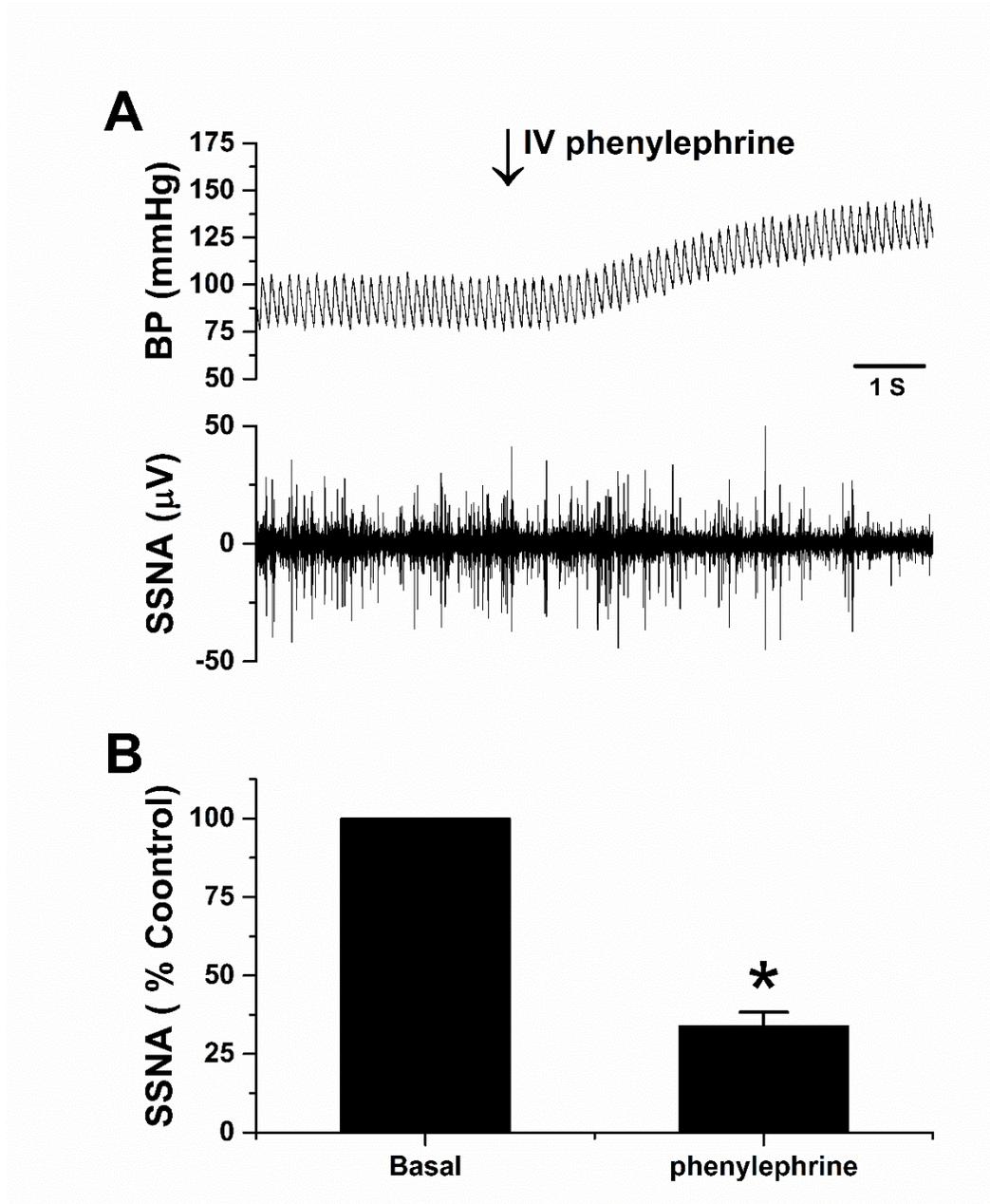


Figure S11. Increases in arterial pressure substantially inhibit, but do not abolish, SSNA. In 7 mice, an iv infusion of phenylephrine (200 $\mu\text{g/ml}$, 10 $\mu\text{L/min}$) increased arterial pressure from 88 ± 3 to 149 ± 7 mmHg ($P < 0.05$) and decreased SSNA to $33.9 \pm 4.4\%$ of control ($P < 0.05$), paired T-tests.

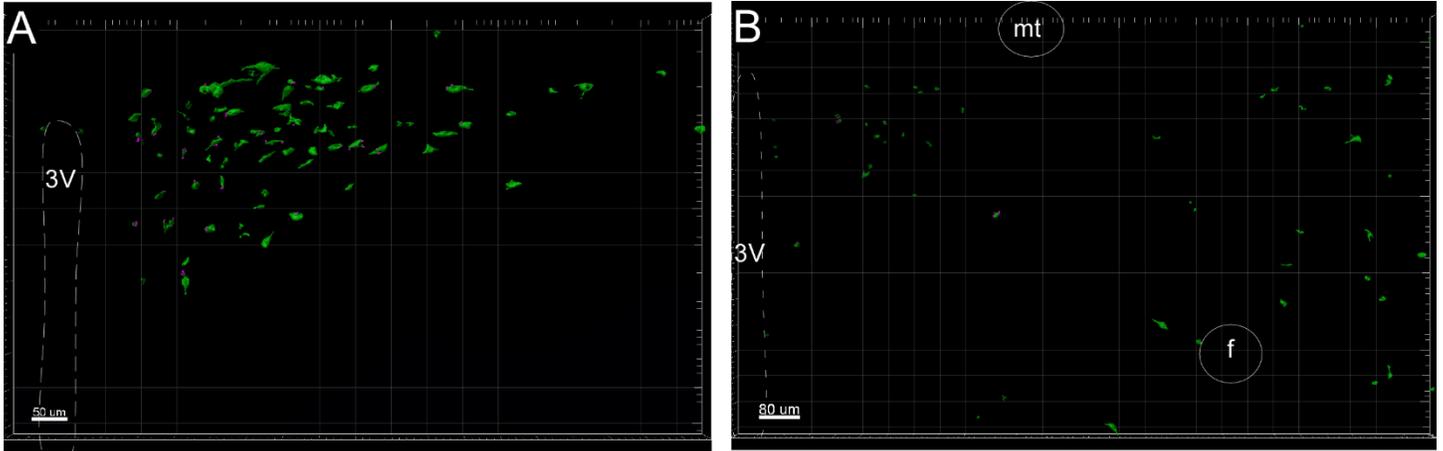


Figure S12. Representative images derived during the quantification of RVLM-projecting neurons with closely associated ArcN NPY/AgRP fibers or terminals. Immunohistochemical labeling of CtB (RVLM-projecting) and mCherry (ArcN NPY/AgRP fibers) processed using Imaris 8.4.1 to create 3-dimensional isosurfaces of the CtB-labeled neurons (pseudo-colored green) and only those mCherry-labeled fibers within 2 μm of a green cell (pseudo-colored magenta spots) in the PVN (A), and the DMH and LH (B). 3V, third ventricle; f, fornix; mt, mamillothalamic tract.

REFERENCES.

Paxinos G. and Franklin, K.B.J. (2001). The mouse brain in stereotaxic coordinates. Second Edition. (New York: Academic Press).