Do neurons involved in the regulation of body weight directly affect breathing?

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Introduction

- Obstructive sleep apnea (OSA) occurs in 40-70% of the obese individuals and is the most common form of sleep disordered breathing
- Obesity leads to neurological changes in the periaqueductal grey (PAG) and the arcuate nucleus (ARC) - both essential brain regions that modulate breathing.

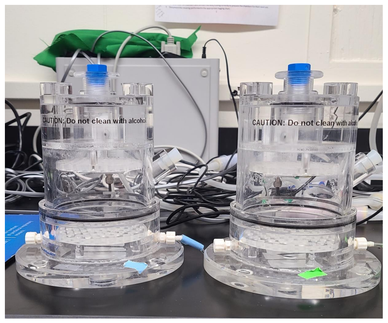
Objective

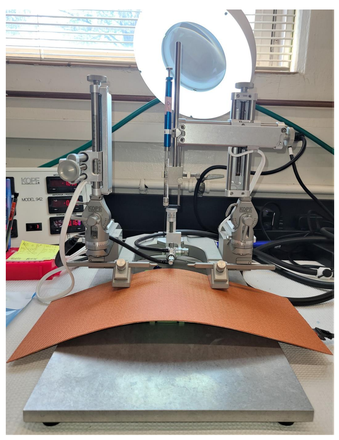
The goal of the project is to determine how a subset of neurons which principally regulate body weight (the ARC_{POMC}) affects breathing.

Methods

Animals. 20-40 weeks old, POMC-Cre (strain number: 005965) and diet-induced obese (DIO) were ordered from Jackson Laboratory (Bar Harbor, ME) and placed on a standard diet (21% Fat, PicoLab 5058) and obesity-inducing diet (60% Fat, Teklad, TD.06414), respectively. Mice were housed in 12L:12D light conditions, with lights on at 8am and lights off at 8pm.

Plethysmography. Baseline ventilation and the hypercapnic ventilatory response (HCVR) was measured in a freely moving, awake, conscious mouse using a whole-body plethysmograph (Emka Technologies, Falls Church, VA). After 30 min acclimation, baseline ventilation is measured while the mice are exposed to room air (e.g. 21% O2, 79% N2, 0% CO2). To determine HCVR, the CO2 concentration inside the chamber is elevated for periods of 4 minutes and separated by 5-minute recovery periods in room air. Mice are exposed to increments of 3%, 5%, and 8% CO2 while real-time ventilatory responses are recorded. As CO2 is increased, N2 is decreased to allow O2 levels to be stabilized at 21%. HCVR is calculated as the slope of the line (min vol/[CO2]).





Stereotaxic Surgery for Designer Receptor Exclusively Activated by Designer Drug (DREADD). For the ARC, 100 nl of virus was injected into an anesthetized mouse at an 8o angle, anteroposterior (AP) -1.5, mediolateral (ML) ±0.8, and dorsoventral (DV) -5.9. Both inhibitory (AAV8.hSyn.DIO.hM4D(Gi)- mCherry) and excitatory (AAV8.hSyn.DIO.hM3D(Gq)-mCherry) DREADDs were ordered from Addgene (Watertown, MA). Four weeks after surgeries, mice were chosen at random to receive either clozapine-n-oxide (CNO) (i.e. the designer drug) via an intraperitoneal (i.p.) injection to activate the DREADDs or the vehicle (normal saline), one hour before the plethysmograph testing was performed.

Brain Removal/ Imaging. Brains were removed from mice and placed in 4% paraformaldehyde overnight. Brains were then placed in a 20% sucrose solution and allowed to sink, for at least 2 days. Brains were rinsed in phosphate buffered solution (PBS) and placed in a small block of OCT embedding compound for cryosectioning. Brains were sliced into 40-µm sections on a cryostat (Leica) and placed into small wells containing phosphate buffered solution PBS, 1x. The slices were then mounted on glass slides and coverslipped with Flouromount-G, with DAPI (Invitrogen). Slides were then imaged on a Nikon confocal microscope. The injection site of the DREADD was determined by the expression of cell bodies that expressed the DREADD tagged with mCherry fluorescence. Only mice with DREADD expression within the target brain region were considered "hits." Mice that did not have DREADD expression within the target brain region were considered "misses" and excluded from analyses.

Statistical Analysis. All stats were performed using Graph Pad Prism 9. All values are reported as means ± SEM. * p<0.05. **p<0.01. ***p<0.001. **** p<0.0001

Results

TARGETED MANIPULATION OF PAG NEURONS REDUCES THE CHEMOSENSITIVITY OF LEAN MICE AND ENHANCES THE CHEMOSENSITIVITY OF OBESE MICE.

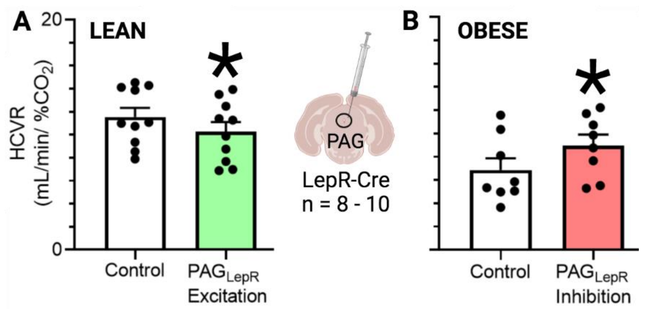


FIGURE 1. Exciting PAG_{LepR} in lean neurons worsens chemosensitivity while inhibiting PAG_{LepR} in obese neurons improves chemosensitivity.

EXCITING ARC_{POMC} NEURONS EXACERBATED THE REDUCED CHEMOSENSITIVITY OBSERVES IN OBESITY.

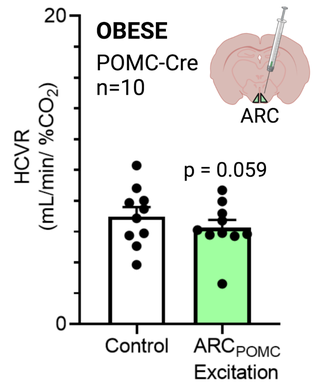
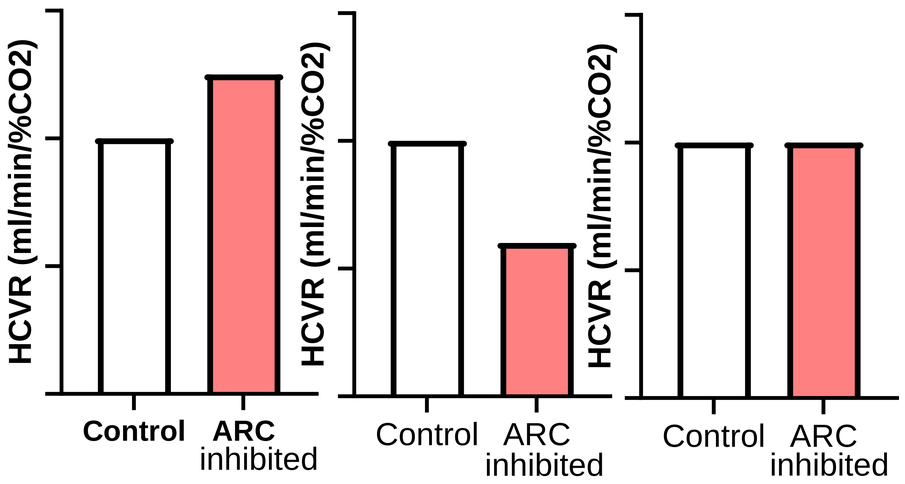


FIGURE 2. Targeted excitation of ARC_{POMC} neurons shows a trend to reduce hypercapnic ventilatory response (HCVR) in obese mice.

POSSIBLE OUTCOMES OF THE HYPOTHESIZED CIRCUIT:



Our hypothesized prediction; removing the excitatory inputs to the PAG restores healthy breathing

Interpretation: The ARC does not excite the PAG, and/or another brain region primarily drives PAG excitation and disordered breathing

ARC is not the primary effector of obesity-associated disordered breathing

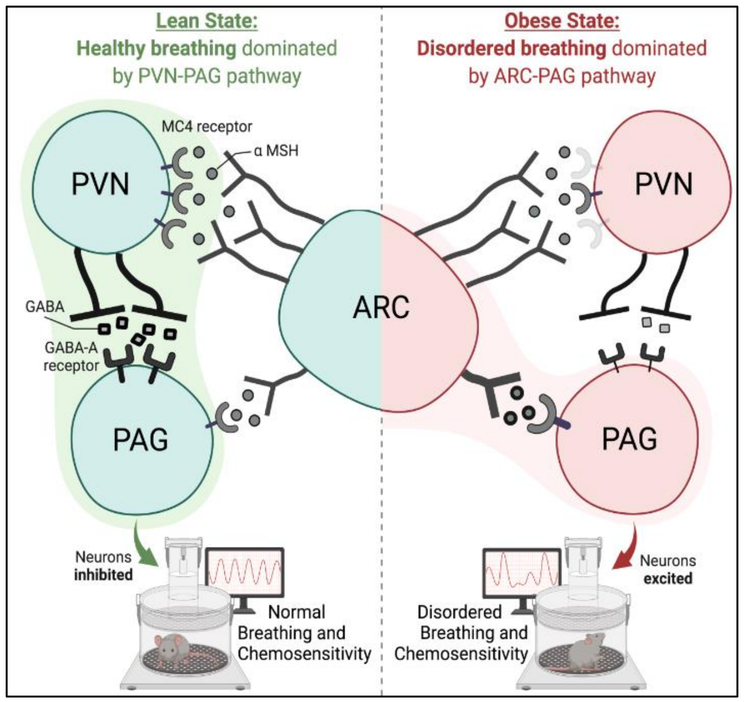


Figure 3. Hypothesized circuit of disordered breathing

Conclusions

- Based on our understanding, we have predicted three different outcomes that could potentially determine the role which the ARC plays in the hypothesized breathing circuit (figure 4).
- If our hypothesis is supported, inhibiting the metabolic ARC brain region may help restore healthy breathing to obese mice.

ACKNOWLEDGMENTS

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