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NEURAL CIRCUITS

# A circuit to sigh for

Sighs are frequently associated with states such as sadness or exhaustion; however, such long, deep breaths also happen spontaneously, potentially to allow collapsed alveoli to be reinflated. How sighing is controlled is incompletely understood, but now Feldman, Krasnow and colleagues identify two central peptidergic pathways that regulate sighing in rodents.

The authors examined more than 19,000 gene expression patterns in the embryonic mouse hindbrain to detect genes that were selectively expressed in regions implicated in breathing control. The most selective pattern belonged to the peptide-encoding gene neuromedin B (*Nmb*), which was expressed in, among other regions, the retrotrapezoid nucleus (RTN), a breathing-related region in the medulla. Supporting these data, mice in which green fluorescent protein (GFP) expression was placed under the control of the *Nmb* promoter and regulatory elements (*Nmb*-GFP mice) showed GFP expression in RTN neurons.

NMB-expressing RTN neurons projected to the preBötzinger complex (preBötC), a region of the ventrolateral medulla that rhythmically signals to initiate normal breaths. Interestingly, many preBötC neurons expressed the NMB receptor (NMBR), suggesting that NMB-expressing RTN neurons signal to the preBötC.

NMB is related to the frog neuropeptide bombesin, and a previous study showed that the latter could be used to increase sighing in rats. Here, microinjection of NMB into the preBötC of anaesthetized rats increased sighing frequency, suggesting that NMB regulates sighing in rodents.

The authors next examined *Nmbr*-knockout mice, which exhibited a reduction in sighing. Moreover, pharmacological inhibition of NMBR in the preBötC of anaesthetized rats reduced the frequency of sighing by approximately 50%. Thus, NMBR activity is required for normal basal sighing.

As the findings described above suggested that other signals also regulate sighing, the authors examined whether gastrin-releasing peptide (GRP), the other mammalian bombesin-like peptide, influences such breathing. In mice and rats, GRP was expressed in a region of the RTN and two other nuclei implicated in breathing, and GRP receptor (GRPR) expression was detected in preBötC neurons. Moreover, knockout of *Grpr* in mice and pharmacological inhibition of preBötC GRPRs in anaesthetized rats decreased the frequency of sighing. Together, these findings suggest that these two neuropeptide-mediated pathways both regulate sighing in rodents.

The authors then examined how these pathways interact. In *Nmb*-GFP mice, the expression of GFP and *Grp* mRNA did not overlap in the RTN; however, the peptides' receptors could both be detected in preBötC neurons. Furthermore, the simultaneous inhibition of NMBRs and GRPRs in the preBötC of anaesthetized rats dramatically decreased or abolished sighing. Thus, these findings suggest that both pathways are necessary for and independently regulate basal sighing.

To explore the functional specificity of the peptide-receptor-expressing preBötC neurons, the authors selectively ablated them by injecting this region with saporin-conjugated bombesin, which triggered cell death following internalization. Five days after the injection, sighing was largely abolished, although other aspects of breathing remained normal, suggesting that these neuronal populations selectively function in the regulation of basal sighing. After this period, however, the mice started to show some disordered breathing, which the authors suggest could be due to a loss of sighing.

Finally, the authors showed that hypoxic conditions, which trigger an increase in sighing in wild-type mice, had no such effect in mice in which NMBR- and GRPR-expressing preBötC neurons were ablated. Thus, these neurons not only mediate basal sighing but also seem to have a role in induced sighing.

Together, these data reveal two peptidergic pathways that regulate basal and hypoxia-induced sighing in rodents but that do not seem to be required for other breathing responses.

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> “microinjection of NMB into the preBötC of anaesthetized rats increased sighing frequency”

**ORIGINAL ARTICLE** Li, P. *et al.* The peptidergic control circuit for sighing. *Nature* **530**, 293–297 (2016)

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