Nature Reviews Neuroscience | Published online 23 Jun 2016; doi:10.1038/nrn.2016.88

BEHAVIOURAL NEUROSCIENCE

On the defensive

Threatening situations or stimuli can trigger the expression of defensive behaviours such as freezing or flight; however, the circuits mediating these behavioural responses are not clear. Lüthi and colleagues now characterize a circuit for the freezing response in mice that involves the ventrolateral periaqueductal grey (vlPAG), and show that this pathway interacts with other circuits that mediate defensive responses.

Previous work has implicated the PAG in defensive behaviours. Here, the authors found that optogenetic excitation of glutamatergic neurons (as revealed by the expression of vesicular glutamate transporter 2 (VGLUT2)) in the vlPAG of mice placed in a novel context triggered freezing, whereas light-induced inhibition of these neurons when a fear-conditioned tone was presented to the mice prevented freezing. Thus, glutamatergic vlPAG neurons are key regulators of the freezing response.

To identify the inputs to glutamatergic vlPAG neurons, the authors injected retrogradely transported fluorescent latex beads into the vlPAG of mice. This approach revealed that the vlPAG is innervated by GABAergic projections from the central nucleus of the amygdala (CeA). Monosynaptic tracing using a rabies virus-based system revealed that these GABAergic CeA neurons preferentially synapse onto GABAergic neurons in the vlPAG. Optogenetic activation of non-glutamatergic vlPAG neurons induced GABA type A receptor ($GABA_AR$)-dependent inhibitory postsynaptic currents (IPSCs) in approximately 50% of recorded glutamatergic neurons. Moreover, extracellular recordings from optically identified neurons in freely moving mice revealed that GABAergic vlPAG neurons are inhibited during freezing. Together, these findings provide evidence for a disinhibitory circuit in which GABAergic CeA neurons inhibit GABAergic vlPAG neurons that, in turn, suppress the activity of the glutamatergic vlPAG neurons that control freezing.

To determine how this circuit stops locomotion during freezing, the authors looked for interactions between glutamatergic vlPAG neurons and neurons that monosynaptically target forelimb motor neurons, and found a population of glutamatergic vlPAG neurons that synapse onto premotor neurons in the magnocellular nucleus of the medulla (McM). In mice in which channelrhodopsin 2 was expressed specifically in McM-projecting glutamatergic vlPAG neurons, light stimulation of these cells robustly induced freezing, suggesting that these cells control locomotion through projections to premotor neurons.

Importantly, McM-projecting vlPAG neurons exhibited $GABA_AR$-dependent IPSCs in response to optogenetic activation of non-glutamatergic vlPAG neurons, suggesting that, in this circuit, glutamatergic outputs to the McM are inhibited by GABAergic vlPAG cells. Consistent with this, optogenetic inhibition and excitation of GABAergic vlPAG neurons increased freezing of mice placed in a neutral novel context and reduced freezing in response to a fear-conditioned tone, respectively.

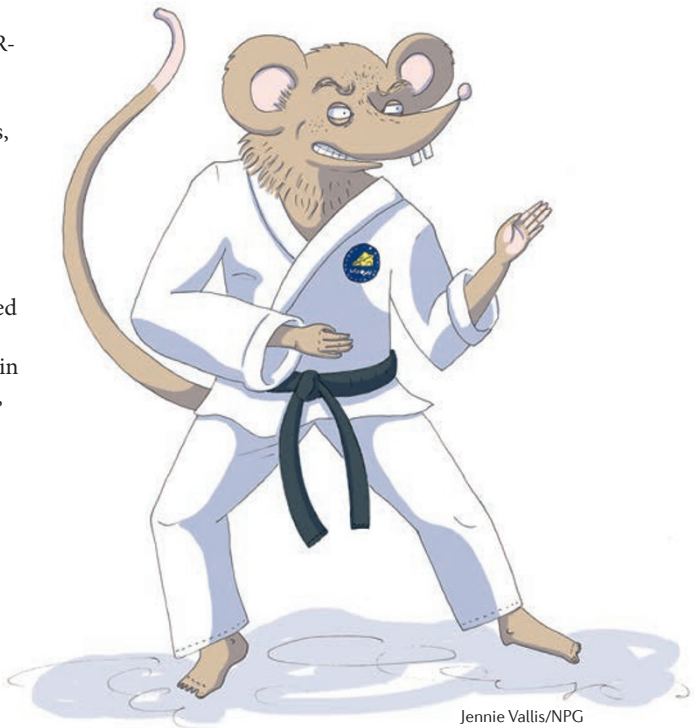
Optogenetic stimulation of glutamatergic neurons in the dorsolateral–lateral PAG (dl/lPAG) resulted in non-freezing defensive behaviours such as flight responses. Thus, the authors considered how dl/lPAG and vlPAG neurons might interact to select individual defensive behaviours. Notably, they identified glutamatergic dl/lPAG neurons that synapse onto a population of GABAergic vlPAG neurons, which, when activated, promoted the flight response. Thus, neurons in the vlPAG may integrate signals from other parts of the PAG to select specific defensive behaviours.

Overall, this study identifies a CeA–vlPAG–McM disinhibitory circuit that mediates freezing, and outlines a possible mechanism for the switching from a freezing to a flight response. The authors suggest that the PAG may integrate various signals to enable the rapid selection of a defensive behaviour in response to threat.

Natasha Bray

> “glutamatergic vlPAG neurons are key regulators of the freezing response”

ORIGINAL ARTICLE Tovote, P. *et al.* Midbrain circuits for defensive behaviour. *Nature* http://www.dx.doi.org/10.1038/nature17996 (2016)
FURTHER READING Tovote, P., Fadok, J. P. & Lüthi, A. Neuronal circuits for fear and anxiety. *Nat. Rev. Neurosci.* **16**, 317–331 (2015)



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