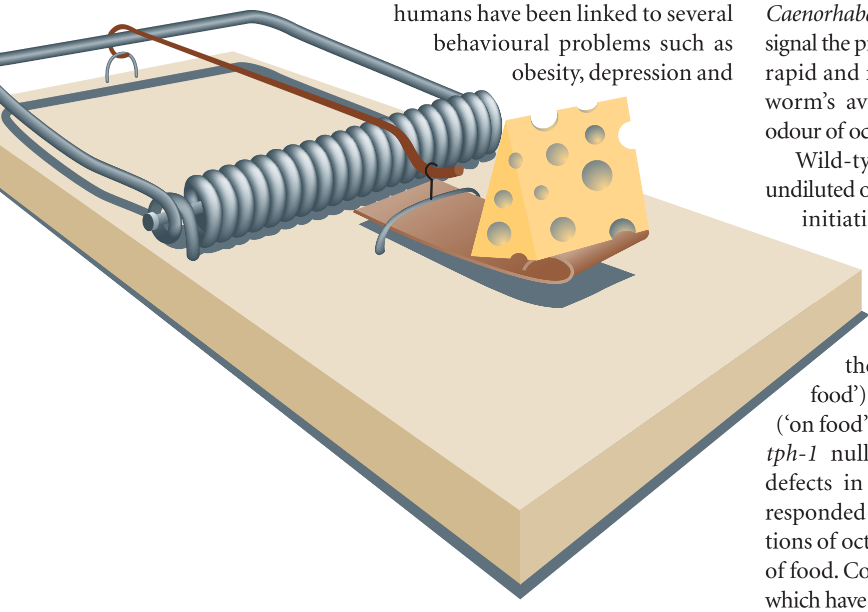
BEHAVIOURAL NEUROSCIENCE

Of food and danger



Defects in serotonin regulation in humans have been linked to several behavioural problems such as obesity, depression and addiction. How serotonin affects human behaviours is unclear, but studies on animals might shed some light on this. Reporting in the *Proceedings of the National Academy of Sciences*, Chao and colleagues show that, in the nervous system of *Caenorhabditis elegans*, serotonin can signal the presence of food and lead to rapid and reversible changes in the worm's avoidance of the noxious odour of octanol.

Wild-type *C. elegans* respond to undiluted octanol in three seconds by initiating backward movement regardless of feeding status. When diluted octanol is used, they respond more slowly in the absence of food ('off food') than when food is present ('on food'). The authors found that *tph-1* null mutants, which show defects in serotonin biosynthesis, responded poorly to low concentrations of octanol even in the presence of food. Conversely, *mod-5* mutants, which have increased serotonin levels, were hypersensitive to diluted octanol off food. Chao *et al.* then removed subpopulations of sensory neurons by laser microsurgery and found that ASH, but not ADL or AWB, neurons are responsible for detecting diluted octanol both on and off food. Serotonin signalling was mediated by a Gα protein encoded by *gpa-11*, as *gpa-11* null mutants responded poorly to diluted octanol on and off food.

Detecting undiluted octanol, however, is more complicated. Although worms can normally sense undiluted octanol equally well on and off food, mutant worms with ASH neurons removed by laser microsurgery fail to respond to undiluted octanol on food. By contrast, the avoidance of undiluted octanol off food can only be abolished when ADL and AWB neurons are also ablated. These data indicate that ASH neurons are mainly responsible for sensing undiluted octanol on food, and that all three types of neurons are involved when

SYNAPTIC FUNCTION

Adapting to epilepsy

AP-3 is a member of the adaptor protein (AP) complex family, which regulates formation of clathrin-coated vesicles and intracellular trafficking of membrane proteins. Although the function of the ubiquitously expressed AP-3A has been elucidated, that of the neuron-specific AP-3B remains unknown. Reporting in *Journal of Cell Biology*, Nakatsu and colleagues show that AP-3B is important in the regulation of GABA (γ-aminobutyric acid) release and might underlie the pathogenesis of epilepsy.

The authors found that mice that lack μ3B, a subunit of AP-3B, have no abnormalities in their overall brain structure. However, these animals showed spontaneous epileptic seizures when presented with a stimulus such as positional change. When intravenous infusion of pentylenetetrazole (a $GABA_A$ receptor antagonist) and electrical kindling were used to trigger seizures, a much lower level of stimulus was required to induce seizures in μ3B-knockout mice compared to their wild-type counterparts, indicating that the mutant animals have higher susceptibility to seizures.

Although the brain morphology of μ3B-knockout mice is basically normal, the number of synaptic vesicles per unit area is lower in the hippocampus, and the diameter of the synaptic vesicles in inhibitory synaptic terminals is also smaller. These observations prompted the authors to test synaptic function and neurotransmitter release in mutant mice. They found that the basal release of glutamate and GABA in hippocampal mini-slices was normal in the mutant mice, but that the K^+-evoked release of GABA, but not of glutamate, was significantly reduced.

As the amounts of these neurotransmitters in the hippocampus are comparable between wild-type and mutant mice, the authors suspected that it might be the trafficking rather than metabolism of GABA that was responsible. As expected, the amount of vesicular GABA transporter (VGAT) was lower than normal in synaptosomal lysates from the hippocampus of μ3B-knockout mice, whereas the concentrations of vesicle glutamate transporters and other synaptic vesicle proteins were normal.

If the inhibitory pathway is weaker due to reduced amounts of VGAT and GABA release on stimulation, are neurons more excitable in μ3B-knockout mice? The authors found that long-term potentiation (LTP) induced by standard conditioning was intact in the mutant mice. When weak conditioning was applied, LTP was induced in the mutant but not wild-type mice. This difference disappeared when the $GABA_A$ antagonist picrotoxin was present, indicating that weaker stimulation can induce LTP in μ3B-knockout mice, because the inhibition is weaker.

This is an interesting finding that assigns a new function to AP-3B and adds another aspect to the pathogenesis of epilepsy. μ3B-knockout mice might serve as a novel animal model of this disorder, which affects millions of people worldwide.

Jane Qiu

References and links

ORIGINAL RESEARCH PAPER Nakatsu, F. *et al.* Defective function of GABA-containing synaptic vesicles in mice lacking the AP-3B clathrin adaptor. *J. Cell Biol.* **167**, 293–302 (2004)

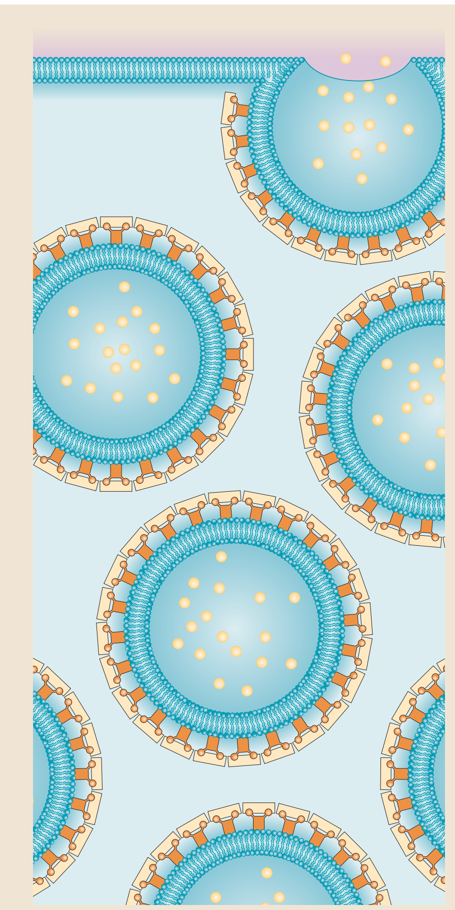
off food. Interestingly, the authors found that serotonin is important under both conditions, although different signalling pathways might be activated depending on feeding status. In the absence of food, serotonin induces avoidance behaviours and requires the postsynaptic glutamate receptor GLR-1. Neither GLR-1 nor GPA-11 is involved in detecting undiluted octanol on food, and Chao and colleagues are still trying to identify the signalling components that are activated by serotonin under this condition.

It is intriguing that worms use different strategies to detect noxious stimuli depending on the strength of the signals and their feeding status. Whether this finding can help to explain serotonin-mediated behaviours in vertebrates remains to be seen.

Jane Qiu

References and links

ORIGINAL RESEARCH PAPER Chao, M. Y. *et al.* Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. *Proc. Natl Acad. Sci. USA* **101**, 15512–15517 (2004)



DEVELOPMENT

Dividing the diencephalon

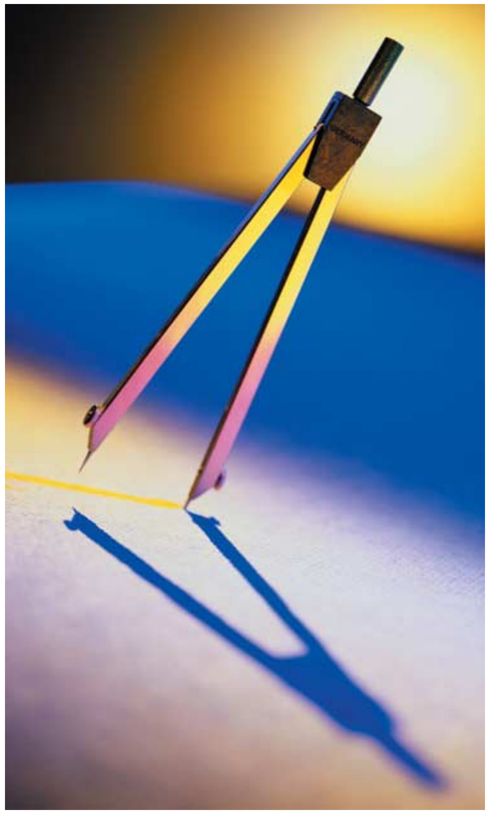
Despite repeated attempts to define the embryonic forebrain as a segmented structure, only two true cell-lineage-restriction boundaries have been identified — the mesencephalic/synencephalic boundary and the zona limitans intrathalamica (ZLI). Reporting in *Nature Neuroscience*, Kiecker and Lumsden show that, as well as physically segregating the prethalamic and thalamic primordia in the diencephalon (the posterior subdivision of the forebrain), the ZLI provides a crucial signal that regulates regional cell identity.

In the early chick brain, the ZLI initially manifests itself as a wedge-shaped gap in the expression domain of the *lunatic fringe* gene. This wedge subsequently collapses into a narrow strip and begins to express the signalling molecule sonic hedgehog (SHH). Kiecker and Lumsden found that SHH-signal transducers and downstream targets of SHH are expressed on either side of the ZLI, confirming that SHH signalling is operational in this area.

Because of the pivotal role of SHH in embryonic development, global *Shh* knockouts in mice have not been informative about its specific role at the ZLI. Therefore, the authors used *in ovo* electroporation to manipulate SHH signalling activity in a spatiotemporally restricted manner in chick embryos, and they examined the effects of SHH gain and loss of function on the expression of various homeobox genes in the diencephalon.

Normally, the thalamus expresses *GBX2*, and is also characterized by the absence of *PAX6* expression, whereas the prethalamus expresses *DLX2* and *PAX6*. Overexpression of SHH in the diencephalon caused an expansion of the *GBX2* domain in the thalamic region and the *DLX2* domain in the prethalamic region. Moreover, *PAX6* was downregulated prematurely in the thalamus, but its expression was unaffected in the prethalamus.

To make diencephalic cells unresponsive to SHH signalling, the authors transfected the diencephalon with a DNA construct that expressed $\text{Ptc}^{\Delta\text{loop2}}$, a mutant form of the SHH receptor Patched. In the transfected embryos, SHH signalling was inhibited in a mosaic fashion, and there were patches of thalamic cells that did not express *GBX2* and patches of prethalamic cells that did not express *DLX2*. Most strikingly, the thalamic cells that expressed $\text{Ptc}^{\Delta\text{loop2}}$ failed to downregulate *PAX6*, although this gene was downregulated in $\text{Ptc}^{\Delta\text{loop2}}$-expressing prethalamic cells. Therefore, SHH seems to have opposing effects on the thalamus and prethalamus with regard to *PAX6* expression.



The prethalamus and thalamus both seem to need SHH signalling to acquire their distinctive gene-expression profiles, but how can this single signal elicit different responses on either side of its source? Part of the answer might lie with the prepatterning gene IRX3, which has its anterior expression boundary at the ZLI. Ectopic expression of *IRX3* in the prethalamic region caused upregulation of thalamus-specific markers, including *GBX2*, and downregulation of *DLX2*. These effects could be rescued by expressing $\text{Ptc}^{\Delta\text{loop2}}$ in conjunction with *IRX3*. Therefore, *IRX3* seems to make diencephalic tissue competent to acquire a thalamic identity in response to SHH.

These findings provide intriguing new insights into patterning mechanisms in the diencephalon, and the ZLI can now be added to the growing list of local signalling centres in the developing CNS.

Heather Wood

References and links

ORIGINAL RESEARCH PAPER Kiecker, C. & Lumsden, A. Hedgehog signaling from the ZLI regulates diencephalic regional identity. *Nature Neurosci.* **7**, 1242–1249 (2004)

FURTHER READING Puelles, L. & Rubenstein, J. L. Forebrain gene expression domains and the evolving prosomeric model. *Trends Neurosci.* **26**, 469–476 (2003) | Gómez-Skarmeta *et al.* Half a century of neuronal prepatterning: the story of a few bristles and many genes. *Nature Rev. Neurosci.* **4**, 587–598 (2003)