

## BEHAVIOUR

# Stitching together a song memory



Photograph of a white-crowned sparrow, courtesy of F. Goller, University of Utah, USA.

New results published in *Nature* show that songbirds can accomplish remarkable feats of learning, at least when it comes to learning to sing. Rather like sequencing a genome by shotgun sequencing — splitting the genome into fragments that can be easily sequenced and then put back in the right order — white-crowned sparrows can learn their songs in pieces.

Shotgun sequencing requires a computer to reassemble the short sequences into the correct order by matching overlapping segments. Rose *et al.* show that white-crowned sparrows are capable of a similar feat when it comes to learning their song: after being exposed only to short but

overlapping snippets of a ‘tutor song’, the young songbirds can reproduce the full tune. Beyond the remarkable animal behaviour story, the results offer insight into how the brain might store auditory memories that guide learned vocal behaviour.

The authors collected nestlings and exposed them to a recorded tutor song from the age of about 10 days. The researchers deliberately fragmented the recordings, so instead of a usual white-crowned song — which consists of four or five ordered ‘phrases’ of whistles, trills, buzzes and so on — the young birds listened to pairs of normally adjacent phrases. For example, if a full song consists of ABCDE (with each letter representing

a phrase), the birds might have heard DE, CD, BC and AB, with silent intervals separating the phrase pairs.

Rose *et al.* found that birds tutored on only adjacent phrase pairs could produce a normal, correctly ordered song, akin to singing ‘Mary had a little lamb’ correctly after only having ever heard ‘little lamb’ ‘a little’ ‘had a’ and ‘Mary had’. Furthermore, in a separate experiment in which birds were tutored with reverse-order syllables (BA, CB, etc.) the birds learned to sing a fully reversed song (EDCBA). This latter result is even more remarkable in light of previous evidence that white-crowned sparrows have a strong innate tendency — even in the absence of any tutor — to start songs with ‘A-like’ introductory phrases. The authors confirmed that the overlapping phrase-pairs were essential, as birds that only heard single phrases — even though they heard all the phrases — could not sing normal songs.

## ION CHANNELS

# Sensing the pressure

Mechanosensitive ion channels convert external mechanical forces into electrical and chemical signals in the cell. In the case of the TREK-1 channel, it is opened by membrane stretch and cell swelling, which results in outward rectifying potassium currents and shaping of the action potential. Reporting in the *EMBO Journal*, Chemin and colleagues found that phospholipids are important in this mechanical gating process.

An artificial increase in the cytosolic concentration of phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) — a component of membrane phospholipids — stimulates the activity of endogenous TREK-1 in cultured striatal neurons. This elevation in channel activity is also seen when the cytosol is acidified, and the effects of both PIP<sub>2</sub> and low intracellular pH can be inhibited by the presence of positively charged intracellular molecules such as polylysine. The inhibitory effect of polylysine molecules is thought to be due to their interaction with the negative charges of membrane phospholipids, which prevents them from interacting electrostatically with specific positively charged segments in the channel protein.

The authors also show that PIP<sub>2</sub> and acid can regulate the gating of TREK-1 in a cooperative

manner: when TREK-1 is challenged with acid after addition of PIP<sub>2</sub>, it becomes ‘locked’ in a highly active form even after the cytosolic pH is returned to pH 7.2. The activity of the locked TREK-1 channel is high even at atmospheric pressure, and pressure stimulation produces only a modest further activating effect. TREK-1 activation becomes more resistant to membrane stretch in the presence of polylysine, which indicates that the cationic molecule interferes with the pressure-sensing process.

Which segment of the channel is responsible for PIP<sub>2</sub> stimulation? The authors generated truncated, chimaeric and point mutations in TREK-1, and analysed the channel activity in cells that overexpressed the mutant proteins. They found that a protein segment of the cytosolic carboxy-terminal domain of TREK-1 is crucial for PIP<sub>2</sub> stimulation. This region contains several positively charged residues that seem to be attractive candidates for binding to phospholipids. A systematic substitution of the positively charged amino acids by alanines, which are neutral, progressively leads to reduced TREK-1 stimulation by PIP<sub>2</sub>. The authors conclude that the cationic cluster is important for stimulation of TREK-1 by phospholipids.

Chemin and colleagues then reasoned that if this positively charged region of TREK-1 is to interact with phospholipids, it must be localized near the plasma membrane. To show this, they used a complementation yeast system, in which a mutant strain fails to grow at the non-permissive temperature of 37 °C unless it is rescued by the exogenously expressed amino-terminal fragment of hSOS, which lacks the membrane-targeting activity. As this fragment of hSOS is only functional when it gets to the plasma membrane, mutant yeast cells are not rescued unless hSOS is fused to another protein or protein segment that interacts with the plasma membrane. The authors show that the cytosolic cationic cluster of TREK-1 brought the hSOS fragment to the plasma membrane and allowed cell growth at 37 °C.

These findings have shed new light on the gating mechanism of the TREK channels, and might also have more general implications for other types of mechanically activated ion channel, as Hirono and colleagues recently suggested for the hair cell channel.

Jane Qiu

## References and links

**ORIGINAL RESEARCH PAPER** Chemin, J. *et al.* A phospholipid sensor controls mechanogating of the K<sup>+</sup> channel TREK-1. *EMBO J.* 2 December 2004 (10.1038/sj.emboj.7600494)

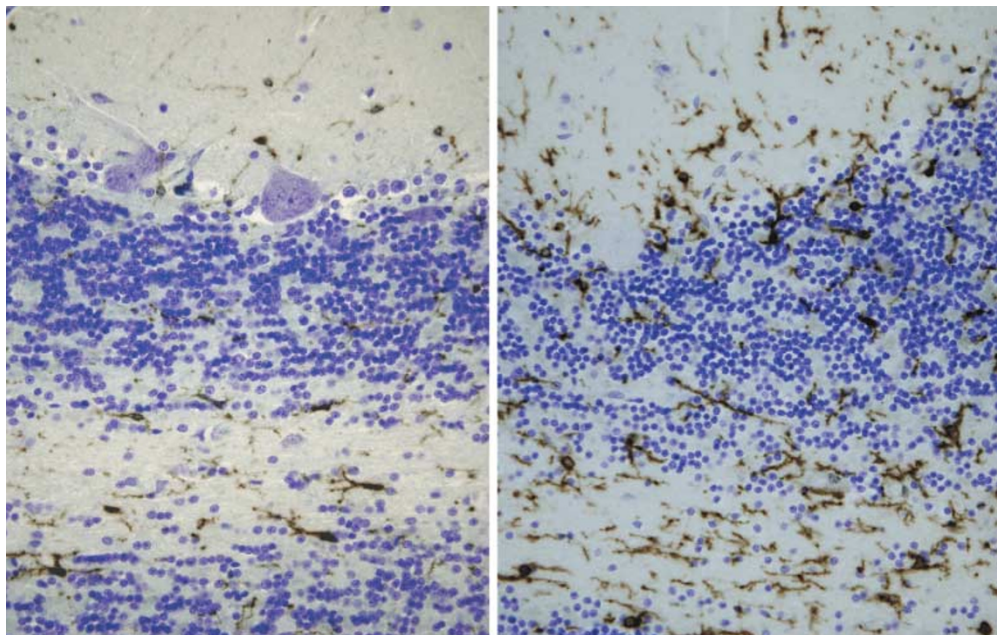
**FURTHER READING** Hirono, M. *et al.* Hair cells require phosphatidylinositol 4,5-bisphosphate for mechanical transduction and adaptation. *Neuron* 44, 309–320 (2004)

During vocal learning a bird forms an auditory memory or 'acquired template' of the tutor song. However, how the nervous system stores such a long-term auditory memory, and how this template guides subsequent vocal learning, has remained largely mysterious. Rose *et al.* now show that the template need not be a representation of the full song. Instead, based on their behavioural data, they propose that a population of phrase-pair detectors might be adequate. Evidence for neurons that might serve as such detectors already exists, so the behavioural results are certain to spur further research into the neural basis of vocal memory.

John Spiro, Senior Editor,  
Nature

### References and links

**ORIGINAL RESEARCH PAPER** Rose, G. J. Species-typical songs in white-crowned sparrows tutored with only phrase pairs. *Nature* **432**, 753–758 (2004)



A marked increase in the number of activated microglia (stained brown) in the cerebellum from patients with autism (right) compared with healthy controls (left). Courtesy of C. A. Pardo, John Hopkins University.

### NEUROLOGICAL DISORDERS

## A new dimension of autism

Autism is regarded as a developmental disorder of the nervous system — an idea that is consistent with the gross abnormalities seen in the brains of patients with autism. The clinical manifestation of the disease is heterogeneous and the causes remain controversial. Reporting in the *Annals of Neurology*, Vargas and colleagues have added another layer of complexity by showing that immune cells are more active in the brains of patients with autism and cause increased inflammation.

Immune dysfunction has been implicated as a potential mechanism for the pathogenesis of autism, but previous research has focused on immunological measures made outside the brain. In this study, the authors analysed pathological changes in brain tissues from 7 healthy controls and 11 patients with autism. They found that, compared with brain tissues from the healthy controls, tissues from the patients with autism showed extensive neuroglial responses in the frontal cortex, cingulate gyrus and cerebellum. These responses are characterized by activation of microglia and astroglia, which are the only immune-competent cells in the nervous system and are important in both innate and adaptive immune responses.

As there was no indication of lymphocyte infiltration or deposits of immunoglobulin and complement in the brain tissues of patients with autism, the immune activity probably reflects the neuroinflammatory reaction associated with the central nervous system's innate immune system. Consistent with this view, there was an increase in the expression of inflammatory cytokines, such as

macrophage chemoattractant protein 1 (MCP1) and transforming growth factor  $\beta$ 1 (TGF $\beta$ 1), in tissue samples from these regions of the brains of patients with autism. Astroglia and, to a lesser degree, microglia, seemed to be the main sources of cytokine production.

These are interesting findings, but immune activity in living patients cannot be detected in this way. The authors reasoned that if there is an ongoing inflammatory reaction in the brains of patients with autism, their cerebrospinal fluid (CSF) might also contain those cytokines. They investigated the immune system of six living patients with autism by measuring the levels of cytokines in their CSF. They found that there was a significant increase in the levels of MCP1 and other cytokines, such as interleukin-6 and interferon- $\gamma$ , in the CSF of patients with autism, whereas the expression of TGF $\beta$ 1 was comparable to that of controls.

Whether these cytokines serve as direct effectors of injury or as neuroprotectants to repair damage has still to be determined. However, these findings have important implications for understanding the pathogenesis of autism and might assist in the diagnosis of this condition.

Jane Qiu

### References and links

**ORIGINAL RESEARCH PAPER** Vargas, D. L. *et al.* Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals Neurol.* 15 November 2004 (10.1002/ana.20315)

**FURTHER READING** Folstein, S. E. & Rosen-Sheidley, B. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nature Rev. Genet.* **2**, 943–955 (2001)

#### WEB SITE

Pardo's laboratory: <http://www.neuro.jhmi.edu/neuroimmunopath>

