MICROGLIA

Awake microglia are less alert

The resident immune cells of the brain, microglia, use their motile processes to survey their surroundings, and are involved in plasticity and responses to brain injury. As most studies of microglial surveillance have been carried out in brain slices or in anaesthetized animals, whether microglia behave differently in awake animals has not been clear. Now, two studies in *Nature Neuroscience* show that microglial surveillance is reduced in awake mice by an increase in noradrenaline (NA) signalling.

Liu et al. and Stowell et al. used two-photon microscopy to track fluorescently labelled cortical microglia in mice during wakefulness and anaesthesia. When mice were anaesthetized, regardless of anaesthetic agent, their microglial processes quickly extended and became more ramified, meaning the surveillance by these processes was increased. Microglia in anaesthetized animals also moved more rapidly to a nearby laser-induced injury site than did the microglia in awake animals. These observations suggest that microglial process surveillance is increased during anaesthesia.

Liu et al. asked whether suppression of neural activity might account for the response of microglia to anaesthesia. The authors reduced local cortical neuronal activity in awake mice by trimming the contralateral whiskers, pharmacologically silencing local neurons or optogenetically activating local interneurons. In each case, reducing local neuronal activity rapidly increased microglial ramification in the same cortical area.

Previous research has shown that NA levels in the cortex are decreased during anaesthesia. Liu et al. found that intracerebral application of NA blocked the increase in microglial process surveillance associated with anaesthesia, contralateral whisker trimming or optogenetic suppression of local neuronal activity. Furthermore, Liu et al. and Stowell et al. respectively found that chemogenetic or pharmacological inhibition of NA release from neurons projecting from the locus coeruleus (LC) in awake mice induces

Credit: Jennie Vallis/Springer Nature Limited

> reducing NA signalling may increase microglial process surveillance

NEURAL DEVELOPMENT

Holding back axon growth

Neuronal polarization, the process by which developing axons and dendrites acquire their distinct structural and functional features, requires the reorganization of the neuronal cytoskeleton; however, the molecular mechanisms that regulate these changes are unclear. Bradke and colleagues now describe a key role for the RHO-family GTPase RHOA in the coordination of cytoskeletal changes during axon growth in mice.

In many CNS neurons, axon formation begins with the selection of one immature neurite to become an axon (axon specification) and is followed by the extension of this 'trailing process' to form the axon. Within the axonal growth cone, interactions between protruding microtubules and the actin-filament network regulate growth. Overexpression studies have suggested a role for RHOA in the control of axon development; however, its precise role in this process has not been defined.

To investigate the potential role of RHOA in axon specification, the authors used in utero electroporation to selectively abolish neuronal RHOA expression in mouse embryos from around the time of axonal specification (embryonic day (E) 13.5). When examined at E15.5, the morphology of neurons lacking RHOA (RHOA KO neurons) was identical to that of wild-type neurons, with each cell possessing a single trailing process. Likewise, fluorescently labelled adult neurons in transgenic mice lacking RHOA expression throughout the brain exhibited single axons, suggesting that RHOA is not required for axon specification.

These findings suggested that RHOA makes a contribution to later stages of axon development, such as axon extension. To examine this possibility, the authors measured axonal growth trajectories in embryos in which neuronal RHOA expression was abolished from E12.5 onwards. By E14.5, the axons of RHOA KO neurons were longer than those of wild-type neurons. Similarly, imaging of axon development in organotypical slice cultures obtained from E13.5 embryos and in dissociated hippocampal neurons obtained from E17.5 embryos confirmed that RHOA KO neurons show more-rapid polarization and faster axonal growth.

> RHOA KO neurons show more-rapid polarization and faster axonal growth

Credit: Jennie Vallis/Springer Nature Limited

a rapid increase in the size of cortical microglial arbors. Thus, reducing NA signalling may increase microglial process surveillance.

Microglia have previously been shown to express β_2-adrenergic receptors (β_2-ARs). Both of the current studies found that blocking β_2-ARs in awake mice increased microglial process surveillance and ramification, mimicking the effects of anaesthesia. Consistent with this, Stowell et al. showed that in anaesthetized mice, β_2-AR agonism caused retraction of microglial processes and reduced the movement of microglia towards laser-injury sites. Therefore, activation of microglial β_2-ARs reduces microglial process surveillance.

Liu et al. found that microglial processes in anaesthetized mice made longer-lasting contacts with neuronal dendrites than did microglia in awake mice, suggesting that changing microglial dynamics could affect plasticity. Indeed, previous work has demonstrated that interfering with microglial signalling can impair ocular dominance plasticity (ODP) — a form of cortical plasticity caused by monocular deprivation. Stowell et al. found that chronically treating monocularly deprived mice with a β_2-AR agonist impaired ODP and reduced microglial contacts with neuronal dendrites. Moreover, deletion of β_2-ARs selectively from microglia protected against the ODP-disrupting effect of the β_2-AR agonist. Thus, aberrant activation of microglial β_2-ARs may disrupt microglial–neuronal interactions and affect plasticity.

Together, these studies provide evidence that microglia behave differently under anaesthesia versus during wakefulness. Moreover, microglial functions — including surveillance of the parenchyma, response to injury and interactions with neurons — are modulated by the stimulation of microglial β_2-ARs.

Natasha Bray

ORIGINAL ARTICLES Liu, Y. U. et al. Neuronal network activity controls microglial process surveillance in awake mice via norepinephrine signaling. *Nat. Neurosci.* **22**, 1771–1781 (2019) | Stowell, R. D. et al. Noradrenergic signaling in the wakeful state inhibits microglial surveillance and synaptic plasticity in the mouse visual cortex. *Nat. Neurosci.* **22**, 1782–1792 (2019)

In combination, the findings point to a role for RHOA in restraining axon extension.

Next, the authors probed the mechanisms that mediate the effects of RHOA on axon growth. Biochemical analyses of cultured RHOA KO neurons revealed a reduction in the activity of myosin II, an actin-binding protein that is activated by one of the key downstream effectors of RHOA signalling. Treating cultured wild-type neurons with the myosin II inhibitor blebbistatin resulted in faster axon growth, whereas overexpression of a constitutively active form of the myosin II regulatory subunit MLC2 reduced axonal growth in RHOA KO neurons. This pointed to myosin II as a key mediator of the effects of RHOA on axon extension.

Myosin II is known to be involved in actin-cytoskeleton remodelling. Actin-filament staining in fixed neuronal cultures, followed by super-resolution microscopy, revealed some important differences between the growth-cone cytoskeletons of RHOA KO neurons or blebbistatin-treated neurons and control neurons. Dense, arc-shaped bundles of actin fibres (known as 'actin arcs') that are thought to prevent the forward protrusion of microtubules were present in the growth cones of most wild-type neurons, but in very few RHOA KO neurons or blebbistatin-treated neurons. The loss of actin arcs in these neurons was accompanied by an increased speed of microtubule advance at the leading edge of the growth cone. Microtubule destabilization via treatment with nocodazole restored axon growth in RHOA KO neurons to wild-type levels.

This study shows that, in mice, RHOA regulates axon extension by promoting myosin II-driven changes in the actin cytoskeleton that restrict microtubule protrusion. As well as enhancing our understanding of a key developmental process, these findings could help us to discover ways to enhance axon regeneration after injury.

Katherine Whalley

ORIGINAL ARTICLE Dupraz, S. et al. RhoA controls axon extension independent of specification in the developing brain. *Curr. Biol.* **29**, 3874–3886 (2019)

IN BRIEF

AUDITORY SYSTEM

Musical pleasure lies in surprise

The basis of musical pleasure in the brain is not clear. Here, participants' ratings of chord pleasantness were found to be predicted by an interaction between uncertainty (lack of prior anticipation) and surprise (deviation from expectation), as quantified using a machine-learning model trained with 80,000 chord progressions. Chords were rated as more pleasant if uncertainty was low and surprise was high, or vice versa. Functional MRI revealed that this interaction modulated responses in the auditory cortex, amygdala and hippocampus.

ORIGINAL ARTICLE Cheung, V. K. M. et al. Uncertainty and surprise jointly predict musical pleasure and amygdala, hippocampus, and auditory cortex activity. *Curr. Biol.* https://doi.org/10.1016/j.cub.2019.09.067 (2019)

GENES AND DISEASE

A protective mutation

Carriers of the *PSEN1*^{E280A} mutation overproduce amyloid-β (Aβ) and develop autosomal dominant Alzheimer disease (ADAD) in their forties. However, this study reports that one woman with *PSEN1*^{E280A} did not show cognitive impairment until her seventies, despite a high burden of Aβ pathology. The woman had two copies of a rare *APOE3* allele with a R136S mutation that reduces APOE3's affinity for heparan sulfate proteoglycans, which have been suggested to promote neuronal uptake of tau. Thus, *APOE3*^{R136S} might delay ADAD by limiting tau pathology.

ORIGINAL ARTICLE Arboleda-Velasquez, J. F. et al. Resistance to autosomal dominant Alzheimer's disease in an *APOE3* Christchurch homozygote: a case report. *Nat. Med.* **25**, 1680–1683 (2019)

BEHAVIOURAL NEUROSCIENCE

A measure of mouse traits

Our understanding of consistent individual differences in behaviour — or 'traits' — in non-human species is limited. Forkosh et al. placed 168 mice in groups of 4 in 'social boxes' and used automatic location tracking to record each animal's behaviour over 4 days. They used a linear discriminant analysis of 60 behavioural dimensions to identify four 'identity domains' (IDs) with high between-individual discriminative ability and high within-individual stability over time, even when mice were placed in different groups. ID scores correlated with scores on multiple behavioural assays and with transcriptomic variance in certain brain regions.

ORIGINAL ARTICLE Forkosh, O. et al. Identity domains capture individual differences from across the behavioral repertoire. *Nat. Neurosci.* https://doi.org/10.1038/s41593-019-0516-y (2019)

NEUROLOGICAL DISORDERS

Hunting out mutant huntingtin

Huntington disease (HD) is caused by the accumulation of mutant huntingtin (mHTT) that contains an expanded polyglutamine (polyQ) tract. Through screening, Li et al. identified four compounds that tether mHTT, but not wild-type HTT, to the autophagy-related protein LC3. The compounds drove autophagy-dependent reductions of mHTT in neurons from a mouse model of HD. Intraperitoneal delivery of two of the compounds reduced cortical mHTT levels in HD mice and improved behavioural deficits in fly and mouse models of HD. Allele-specific linker compounds might have potential in treating HD and other polyQ disorders.

ORIGINAL ARTICLE Li, Z. et al. Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds. *Nature* **575**, 203–209 (2019)