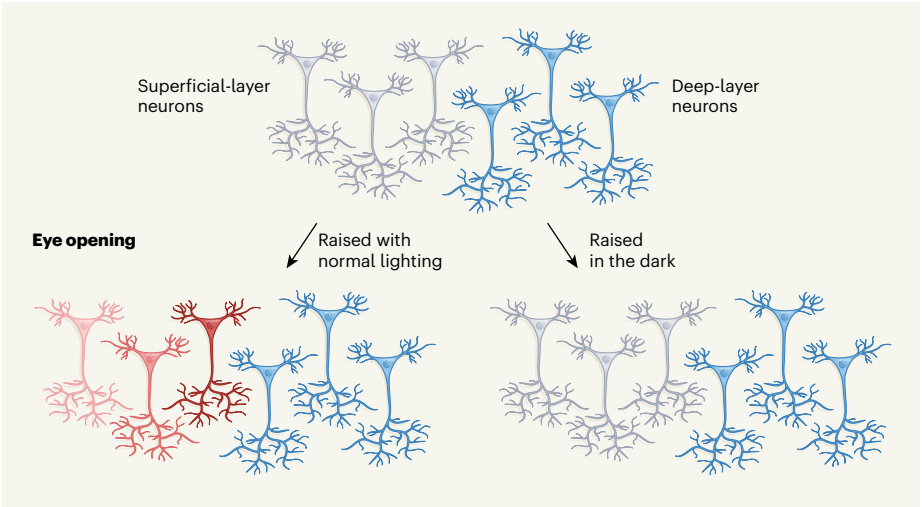

Figure 1 | Vision-dependent neuronal maturation is cell-type specific. The brain's visual cortex consists of two main classes of cell: deep-layer neurons, which connect with deep-brain structures; and superficial-layer neurons, which connect with one another within the cortex. Cheng *et al.*[4] analysed the development of these cells in mouse pups around the time of eye opening. They show that deep-layer neurons are mature (indicated in blue) before mice open their eyes. If the mice are raised in normal lighting, superficial-layer neurons diversify into distinct molecular subtypes (red) after eye opening. By contrast, these neurons fail to diversify in mice raised in the dark. Deep-layer neurons are unaffected by lighting conditions.

of genes in single cells. This allowed the researchers to pinpoint input-dependent gene expression by comparing superficial-layer neurons in dark-reared and light-reared pups. They found that expression of the gene *Igsf9b*, which encodes a cell-surface molecule involved in the formation of inhibitory synaptic connections to neurons, was significantly decreased in dark-reared mice. Cheng *et al.* found that, in mice genetically engineered to lack this gene, subsets of superficial-layer visual cortical neurons showed impaired maturation. This result highlights a key role for synaptic inhibition in the specification of this cell type.

Together, Cheng and colleagues' work reveals that cortical neurons can be unexpectedly diverse in their plasticity. How this variation comes about is unclear, but early developmental processes might offer a clue. During embryonic development, cortical neurons are not born simultaneously. Instead, deep-layer neurons are born first, followed later by superficial-layer neurons[6]. So, at eye opening, superficial-layer neurons might be more genetically malleable than deep-layer neurons, which might have passed through their own malleable stages before eye opening occurred. It follows, then, that deep-layer neurons might serve more hard-wired functions, and that superficial-layer neurons might be better able to modulate their connectivity, allowing behavioural flexibility in response to environmental changes.

The neurons at the centre of this study send excitatory signals, but the work also reveals a role for inhibitory inputs in their maturation — a finding consistent with previous studies on the importance of inhibitory inputs in adult neuronal plasticity[8–10]. Cheng and colleagues did not find evidence that gene expression in inhibitory neurons known as interneurons was affected by light deprivation, but it is possible that the morphological, electrophysiological or circuit properties of these neurons were modified in ways not detectable by single-nucleus RNA sequencing. Likewise, how altered gene expression affects these cellular properties in superficial-layer neurons remains to be discovered.

Whether the environment has a one-size-fits-all role in neuronal specification, or a tailored and cell-type-specific role, has never been directly assessed. Cheng and colleagues reveal that it is the latter that describes how external stimuli affect brain circuits. By nature, it seems, not all neurons can respond to nurture's call.

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The authors declare no competing interests.
This article was published online on 18 February 2022.

Microbiology

Lung microbes mediate spinal-cord autoimmunity

Aubrey M. Schonhoff & Sarkis K. Mazmanian

Lung bacteria modulate the activity of immune cells in the central nervous system in a rodent model of autoimmunity. This finding might shed light on the neuroinflammation associated with multiple sclerosis. **See p.138**

Chronic, uncontrolled inflammation of healthy tissues can lead to damage and autoimmune disease. There is growing evidence that both autoimmunity and the development of normal immune responses in humans are linked to the microbiome — the community of trillions of microorganisms that colonize body surfaces. Most research so far has focused on bacteria living in the gut, with microbial communities in the colon being the most diverse and abundant. There is evidence[1–3] that interactions between the microbiome and the brain have a role in some brain disorders and in complex behaviours such as sociability, although most such studies have focused on the gut–brain axis in animal models.

On page 138, Hosang *et al.*[4] identify a previously unknown effect of the lung microbiome on microglia, the main immune-cell type in the central nervous system (CNS). The authors find that specific lung-resident bacterial species and some of the molecules they produce modify neuroinflammation and associated symptoms in a rat model of autoimmunity. Their result builds on other findings that lung–brain interactions affect the immune response[5,6].

In humans, the gut microbiome is the largest

microbial community[7], and its composition affects immune development and function throughout the body. In a rodent model of multiple sclerosis called experimental autoimmune encephalomyelitis (EAE), the animals have abnormalities resulting from inappropriate activation of immune cells called effector T cells. These cells respond to fragments (termed antigens) of CNS proteins such as myelin basic protein and myelin oligodendrocyte glycoprotein, and mediate autoimmune targeting of the CNS. Many studies have linked the gut microbiome to the progression of EAE in mice (for example, refs 1–3).

By contrast, the bacterial community in the lungs has stayed mainly out of the spotlight. The lungs harbour a less-diverse and numerically smaller microbial community than that in the gut, although lung bacteria have a key role in regulating local immune responses, including those to respiratory infections and in asthma[5]. Furthermore, effector T cells mature in the lungs before migrating to the CNS to trigger autoimmune disease[6].

To investigate the role of the lung microbiome in CNS autoimmunity (Fig. 1), Hosang *et al.* treated rats with low doses of the antibiotic neomycin, delivered directly into the lung; this led to subtle shifts in the composition of the lung microbiome, with no effect on the gut microbiome. Remarkably, this treatment lessened disease symptoms in the EAE model animals. The finding emboldened the authors to explore whether microbial signalling to local immune cells affects CNS inflammation. They found that there was no difference in lung T-cell activation between the neomycin and control (no antibiotic) groups, indicating that the antibiotic was not directly affecting T cells in the lungs. However, the treatment led to fewer T cells infiltrating the CNS. The authors saw similar effects in another rodent model of CNS autoimmunity, in which T cells target the brain protein β-synuclein.

The finding that no changes occurred outside the CNS was unexpected, given that previous work[3] had shown that, in EAE, the gut microbiome can activate T cells locally and affect their differentiation there. Comparative studies are needed to determine whether overlapping or distinct mechanisms mediate the effects of the lung and the gut on neuroinflammation.

For T cells to exit the bloodstream and reach the CNS, adhesion molecules on T cells must first bind to cells lining blood vessels. The T cells then interact with immune cells called antigen-presenting cells on entering the brain or spinal cord[8], and become 'reactivated'. The authors report that neomycin-treated and control animals expressed similar levels of adhesion molecules on T cells circulating in the blood, and that similar levels of pro-inflammatory molecules called cytokines were produced by T cells in the CNS. These findings suggest that these adhesion molecules and T-cell-produced cytokines needed for entry of T cells into the CNS and their reactivation are not affected by antibiotic treatment of the lungs.

Given the lack of effect of neomycin treatment on these factors needed for effector T-cell entry into the CNS, the authors investigated whether the treatment affected neuroinflammation and, specifically, microglia, the major antigen-presenting cell of the CNS (antigen-presenting cells can be involved in triggering autoimmunity). Microglia from rats given neomycin produced fewer cytokines and expressed lower levels of activation markers than did the microglia of control animals. Furthermore, the response profile of microglia from neomycin-treated animals was shifted to favour signalling mediated by the type I interferon pathway. Type I interferon proteins have a protective effect in EAE that is mediated through signalling by cells such as microglia[9]. Hosang and colleagues' data indicate that a type I interferon signature found in microglia, and affected by the lung microbiome, might interfere with the development of neuroinflammation. Indeed, microglia have a role in long-lasting memory of inflammatory stimuli that can alter the course of disease in the CNS[10].

The authors then delved into how neomycin treatment affected the composition of the lung bacterial community. Rats with EAE received lung transfers of *Prevotella melaninogenica*, a member of a group called Bacteroidetes, the bacterial phylum whose presence increased the most substantially on neomycin treatment. Both live and inactivated *P. melaninogenica* protected against EAE symptoms, suggesting that preformed bacterial molecule(s) mediate the beneficial effects observed.

Lipopolysaccharide (LPS) is a component of the bacterial cell wall that can induce type I interferon responses[9]. Hosang *et al.* report that administration of an LPS-neutralizing molecule into the lungs exacerbated disease in animals with EAE, whereas direct administration of LPS into either the lungs or the brain ameliorated disease. These findings are further evidence that LPS outside the CNS can attenuate microglial reactivity[9]. The authors conclude that neomycin treatment of the lung results in higher production of LPS by an

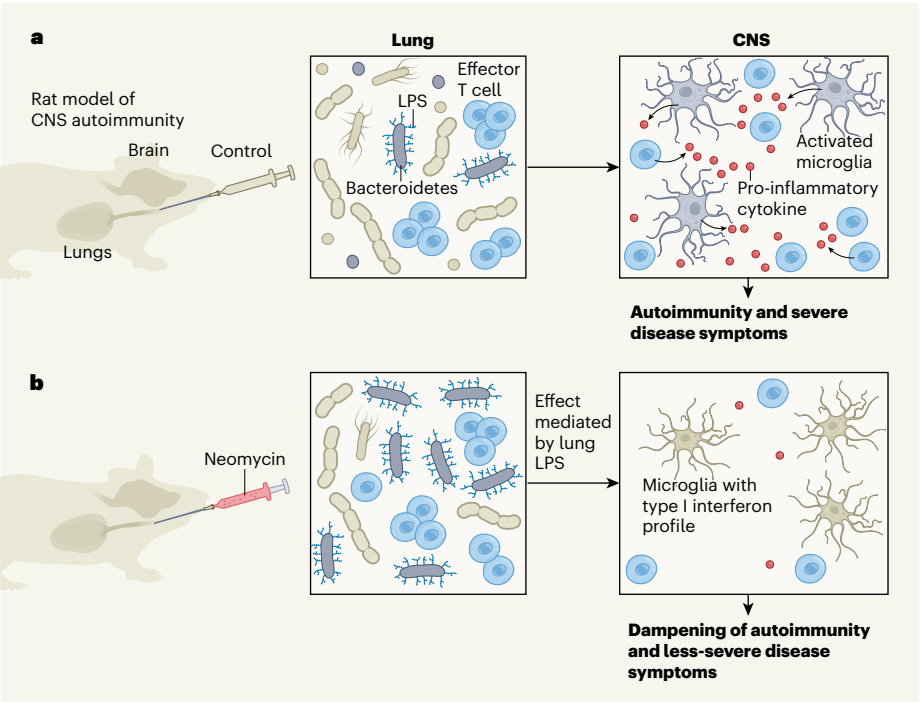

Figure 1 | Lung bacteria modulate autoimmunity in the central nervous system (CNS). Hosang *et al.*[4] investigated the role of the bacterial community in the lung in rat models of CNS autoimmunity, including a model of multiple sclerosis called experimental autoimmune encephalomyelitis. The authors perturbed the bacterial community by direct delivery of the antibiotic neomycin. **a**, The control animals received phosphate-buffered saline instead of neomycin, and the diverse bacterial community in the lungs included some microbes of a bacterial phylum called Bacteroidetes. Such bacteria are coated with the molecule lipopolysaccharide (LPS). The lungs also contain immune cells called effector T cells, which migrate to the CNS and release pro-inflammatory molecules called cytokines. In the brains of these control animals, immune cells called microglia were activated and also released pro-inflammatory cytokines. This autoimmune response caused disease. **b**, Neomycin treatment drove a shift in the lung bacterial community, and the resultant rise in LPS was associated with changes in the brain, including reduced numbers of T cells, lower cytokine levels (due to a decrease in cytokine production by microglia), and microglia with a different response profile (a type I interferon signature). These neomycin-treated animals were healthier than the control rats.

altered lung microbiome, and that this skews microglia towards a type I interferon response, thereby attenuating the development of EAE. Further research should determine whether other bacterial molecules also provide an immunomodulatory effect. Whether this phenomenon occurs and is protective in human autoimmune disease is unknown, but is clearly a question of considerable importance.

Hosang *et al.* present evidence that lung microbiome–microglia interactions affect the severity of CNS autoimmune disease in a rodent model. However, it is unclear whether people with multiple sclerosis have abnormalities in their lung microbiome. Further mechanistic studies are warranted to discover whether the microglial characteristics observed in response to lung neomycin treatment could cause detrimental hyper-reactivity in response to a challenge, such as viral infection, that induces type I interferon responses.

Research is also needed into whether the increased levels of LPS from the lungs reach the CNS and affect microglial activity directly. Indeed, the gut is probably a more abundant source of LPS. It is conceivable that a particular structure or modification of LPS, which can vary in different bacterial species, contributes to altered immune-cell responses[11].

Some studies in mice have directly implicated gut bacteria in EAE[1,2], whereas others have not investigated whether there are differential contributions from the gut and lung microbiomes[3]. Previous work[12] has indicated that interactions between microglia and T cells are not required for the development of EAE; rather, other immune cells, such as dendritic cells and macrophages associated with the blood–brain barrier, can mediate T-cell reactivation in the CNS. The relative effects of gut and lung microbiomes on neuroinflammation should therefore be clarified, as should the roles of microglia in the response to microbial signals from either site.

"The bacterial community in the lungs has stayed mainly out of the spotlight."

Our understanding of how the microbiome affects the immune system and CNS autoimmunity has advanced rapidly in the past decade, making this a promising area for future exploration. Further studies will be required to reproduce and extend Hosang and colleagues' innovative work, and to investigate whether interactions between the lung microbiome and microglia affect other conditions involving inflammation of the CNS, such as neurodegeneration and stroke. For now, this result adds to the enthusiasm over the exploration of brain–body interactions, and unveils ideas about how lung-resident bacteria and their associated molecules might contribute to neuroinflammation.

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The authors declare no competing interests.
This article was published online on 23 February 2022.