as a base and allowed the cracks to be stitched back together with ^{12}C-graphene to create a localized patch of isotopic heterogeneity. The regions in which the cracks healed were only a few nanometres wide, and each contained a sharp interface between ^{12}C and ^{13}C isotopes. This inspired methodology can rigorously and reproducibly generate samples on which nanoscale isotopic analysis can be performed using monochromated electron energy-loss spectroscopy in a scanning transmission electron microscope (Fig. 1b).

There are also challenges associated with the use of graphene: the signal from such a thin sample is extremely low, and the vibrational shifts are three times smaller than the energy resolution of the instrument. The authors overcame these limitations through careful data analysis and thorough validation. By modelling the potential vibrations in graphene, they were able to unambiguously distinguish the ^{12}C-graphene vibrational frequency on the ^{13}C background. Moreover, they eliminated defects as a potential cause of the measured shift by performing control tests on ^{13}C-graphene flakes that had a high concentration of defects, and on ^{12}C-healed cracks on ^{12}C-graphene bases, neither of which produced a shift comparable to the one measured at the isotopic interface.

More crucially, Senga et al. performed the measurement multiple times on the same sample, which offered unique insight into isotopic dynamics in graphene. When they heated the sample at high temperatures for two hours and then repeated the measurement, they observed that the localized domain of ^{12}C had vanished — the isotopes had diffused into the surrounding material and only the ^{13}C signal remained. This observation allowed the authors to directly measure the diffusion of carbon atoms in the homogeneous graphene lattice. Previously, such measurements have been limited to materials with defects and impurities[14], but vibrational electron energy-loss spectroscopy enables isotopic labels to be tracked in a pristine crystal.

Senga and colleagues' work signals the advent of nanometre-scale isotopic analysis in the electron microscope. It has major implications for the study of biological materials in which isotopic labels are used to track chemical-reaction pathways, because it brings unprecedented length scales to these tried-and-tested techniques. Furthermore, the technique opens avenues for exploration using electron microscopy, such as the study of heterostructures consisting of materials containing different isotopes, or in situ nanoscale isotopic tracking during heating or application of an electric voltage.

Jordan A. Hachtel is at Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA.
e-mail: hachtelja@ornl.gov

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Neurobiology

Visual identity isn't a light decision for all neurons

Sergi Roig Puiggros & Denis Jabaudon

When mouse pups first open their eyes, what they see shapes neuronal connectivity. A study shows that this visual experience has cell-type-specific effects, acting only on a subset of malleable neurons.

Environmental stimuli have a crucial role in shaping a developing organism's behaviour. Birds readily accept foster parents — even human ones — provided they see them at birth[1]. Cats raised in an environment consisting only of vertical stripes are later unable to see horizontal lines[2]. Even (brainless) bacteria must react to changes in environmental lactose to keep on thriving and dividing[3]. Distinguishing inborn (nature) from acquired (nurture) facets of development is thus a fundamental quest in biology, and particularly so in the developing brain. But, despite decades of research, we do not understand precisely how the environment shapes the brain at the molecular, cellular and circuit levels. Writing in Cell, Cheng et al.[4] take on this challenge by investigating how postnatal visual experience affects distinct neuronal cell types in the maturing mouse brain.

Mouse pups open their eyes and start exploring their environment at around two weeks of age. Cheng and colleagues collected cells from the brains of mice at 6 time points between 8 and 38 days after birth. The group focused on the primary visual cortex — a part of the brain that integrates information coming from the eyes. They isolated thousands of cell nuclei from this region in each animal and sequenced their RNA, which codes for the cells' proteins. They then used advanced bioinformatics to classify the cells on the basis of the types of gene that they expressed[5], producing a cell atlas for the primary visual cortex.

Cortical neurons are organized into layers. Deep-layer neurons connect deep within the brain to reach the spinal cord, whereas superficial-layer neurons interconnect to form intracortical networks that integrate sensory and motor information[6]. Cheng and colleagues' atlas indicated that neurons in the superficial layer refine their identities after birth, whereas deep-layer neurons do not.

Next, the authors asked how being reared in the dark would affect the development of these visual cortical neurons. The researchers kept mice in a dark environment from 21 to 28 or 38 days of age (a period essential for vision-dependent brain development[7]). They then compared the gene-expression profiles of light- and dark-reared mouse pups. They found that different types of visual cortical neuron have different sensitivities to visual experience. Some cell populations relied on ocular input to mature and differentiate, whereas others were unaffected by an absence of light in the environment.

Specifically, Cheng et al. showed that neurons in the superficial layer are particularly susceptible to visual input. In the absence of light, superficial-layer neurons remained immature, such that crisply defined cell subtypes failed to emerge. By contrast, deep-layer neurons remained impervious to visual deprivation and matured adequately (Fig. 1). The authors found that re-exposure to light after ten days of darkness partially restored the maturation of superficial-layer neurons. This indicates that lack of visual stimulation does not irreversibly jam neuronal specification; rather, neuronal identity remains sensitive to light over long periods of time.

Single-nucleus RNA sequencing provides information about the expression of thousands
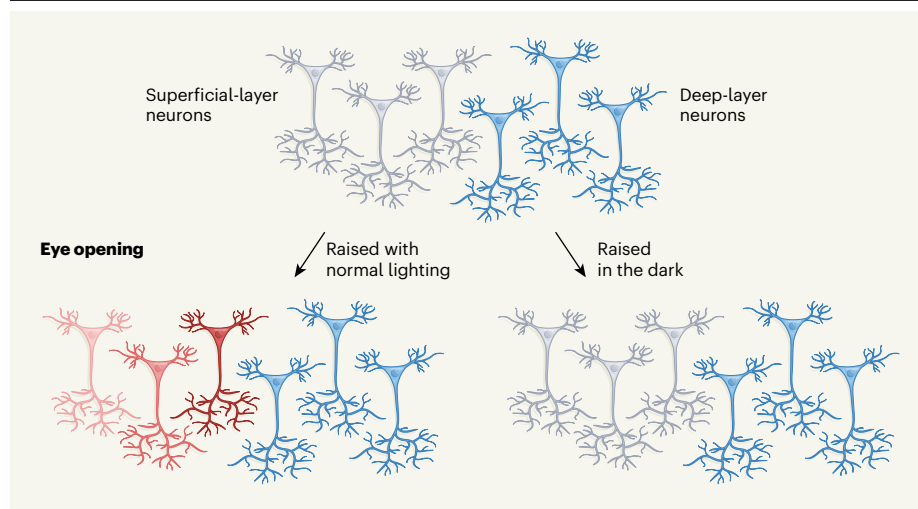
Figure 1 | Vision-dependent neuronal maturation is cell-type specific. The brain's visual cortex consists of two main classes of cell: deep-layer neurons, which connect with deep-brain structures; and superficial-layer neurons, which connect with one another within the cortex. Cheng *et al.*[4] analysed 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, electro-physiological 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.

Sergi Roig Puiggros and **Denis Jabaudon** are in the Department of Basic Neurosciences, University of Geneva, 1211 Geneva, Switzerland. **D.J.** is also in the Department of Clinical Neurosciences, Geneva University Hospital, Geneva.
e-mails: serge.roigpuiggros@unige.ch; denis.jabaudon@unige.ch

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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