

# Reverse engineering the mouse brain

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**Behaviour is governed by activity in highly structured neural circuits. Genetically targeted sensors and switches facilitate measurement and manipulation of activity *in vivo*, linking activity in defined nodes of neural circuits to behaviour. Because of access to specific cell types, these molecular tools will have the largest impact in genetic model systems such as the mouse. Emerging assays of mouse behaviour are beginning to rival those of behaving monkeys in terms of stimulus and behavioural control. We predict that the confluence of new behavioural and molecular tools in the mouse will reveal the logic of complex mammalian circuits.**

A central goal of modern neuroscience is to link the dynamics of neural circuits to behaviour. The operational principles of a neural circuit must be deduced through analysis of its structure and function, a process generically referred to as reverse engineering. Here we advocate a programme of reverse engineering with the aim of linking patterns of action potentials in distinct nodes of neural circuits to animal behaviour.

The circuit diagram, depicting the connections between defined groups of neurons, is necessary to decipher the function of neural circuits. The first step in reverse engineering is the enumeration of the elemental components of the circuit, the cell types<sup>1</sup>, typically on the basis of dendritic and axonal structure. Cell types often express distinct patterns of genes, which potentially provide access to these cell types by transgenesis<sup>2,3</sup> (Fig. 1). In some regions of the brain, such as the retina and the cerebellum, the major cell types are known. In other regions, such as the cerebral cortex, discovery of cell types is ongoing. Each cortical layer corresponds neatly to distinct sets of neurons<sup>4</sup> (Fig. 1). However, each layer harbours multiple intermingled cell types; the total number of neocortical cell types and their relative proportions remain to be determined.

The second step in reverse engineering requires mapping the connections between cell types. In some structures, such as the cerebellum, the connectivity at the level of cell types is well understood. Neuroanatomists and electrophysiologists have measured the connections between the major types of neuron in the neocortex on the basis of position<sup>5–7</sup>. For example, in brain slices of the mouse barrel cortex, the functional connections between excitatory neurons in different layers have been mapped<sup>5</sup> (Fig. 1a). Although this circuit diagram is only a rough draft (see refs 8, 9 for evidence of fine-scale specificity beyond cell types), it reveals that neocortical cell types are connected into orderly circuits.

Because neural circuits have evolved to support specific types of behaviour, the functional logic of neural circuits can probably only be determined in the context of behavioural experiments that engage the circuits of interest. The third step in reverse engineering therefore requires recording the activity of specific cell types in behaving animals. Measurements of spiking activity in behaving subjects are typically made with extracellular recordings of single units<sup>10–12</sup>. In these measurements, the cell type and the unit's relationship with the underlying neural circuit are poorly defined. Responses from all types of neuron are mixed together (however, see refs 10, 13). In addition, neurons are detected on the basis of brisk responses to selected stimuli. These and other factors cause strong sampling biases, a problem that was recognized by early investigators in the field<sup>12,14</sup> but is often underappreciated. Perhaps as a consequence, current network models of cortical activity can afford to ignore information

about cortical cell types and connectivity and represent all cortical neurons by a few simple cell types, connected in generic ways<sup>15,16</sup>. Assigning patterns of activity to defined nodes in the circuit diagram requires cell-type-specific methods for neurophysiology.

Measurements of neural activity naturally lead to models of the computations performed by neural circuits<sup>15–17</sup>. However, inferring design principles from measurements of neural activity alone is treacherous<sup>18</sup>. Hypotheses ultimately need to be tested by manipulating neural activity during behaviour. The fourth step in reverse engineering therefore requires inactivation and activation of specific cell types. Classical experiments involving lesions (reversible and permanent) and electrical microstimulation have led to major discoveries in neuroscience, for example about the neural basis of movement<sup>19</sup> and perception<sup>20</sup>. However, these methods lack cell-type specificity and reveal relatively little about circuit-level mechanisms, except in unusual circumstances<sup>21,22</sup>. Routine manipulation of defined nodes of the circuit diagram will require cell-type-specific methods for controlling neural activity.

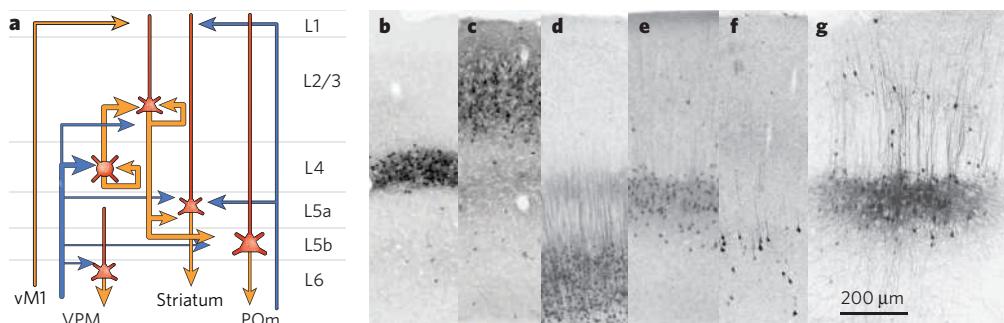
Molecular tools to interrogate and manipulate defined cell types in intact circuits, which have long been advocated<sup>23</sup>, are now becoming a reality<sup>3</sup>. These tools will allow reverse engineering of neural circuits with the precise goal of linking patterns of activity in specific cell types, within well-defined neural circuits, to animal behaviour. Because of the power of genetic methods, these tools are most amenable to use in genetically tractable organisms. Among mammals, the mouse holds a special place as the only established genetic model system.

Here we review recent progress in the deployment of these molecular tools in behaving mice. We discuss advances in mouse behavioural experiments with excellent stimulus control, quantitative behavioural measurements and repeatability. We argue that the confluence of molecular methods for circuit analysis, genetic targeting in mice and quantitative mouse behaviour will help to establish causal relationships between patterns of action potentials in specific neuronal populations and behaviour. Beyond the analysis of cell types, it may be possible to directly explore the function of heterogeneous response types within a single class of neuron. The examples we consider focus on neocortical mechanisms of perceptual behaviour in mice, reflecting space limitations and our research interests, but the concepts and techniques we discuss are applicable to other problems in genetic or, with limitations, non-genetic model organisms.

## Molecules for cell-type-specific physiology

Protein sensors and switches are beginning to revolutionize neuroanatomy and neurophysiology in genetic model organisms (see ref. 3 for a discussion of the complex technical issues involved in the use of

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**Figure 1 | Major excitatory pathways in the mouse somatosensory cortex and genetic access to the cell types.** **a**, Circuit diagram<sup>5,55</sup>. Blue, ascending thalamocortical input from the ventral posterior medial nucleus (VPM) and the medial subdivision of the posterior nucleus (POm); orange, intracortical (including from whisker-related motor cortex (vM1)) and descending projections; red, dendritic arborizations. **b–g**, Sections through the somatosensory cortex of bacterial artificial chromosome (BAC)-transgenic mice: Scnn1a-Tg3-Cre driver line crossed with reporter mice, labelling L4 stellate cells (**b**); Wfs1-Tg2-Cre driver line crossed with reporter mice, labelling L2/3 pyramidal neurons (**c**);

these methods). Protein sensors convert changes in neural state (for example membrane potential,  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]$ ), exocytosis, protein–protein interaction) to changes in fluorescence signals. Genetically encoded calcium indicators are particularly promising for neurophysiology because they efficiently monitor cytoplasmic  $[\text{Ca}^{2+}]$  changes induced by trains of action potentials<sup>3</sup>, but genetically encoded voltage sensors are catching up<sup>24</sup>. Imaging populations of neurons expressing protein indicators may allow measurement of activity in multiple — perhaps all — neurons of a particular type over time<sup>25</sup> (Fig. 2).

The development of genetically targeted molecular switches has made possible the manipulation of activity in defined cell types in intact mice with relatively high temporal and spatial precision<sup>3</sup>. Protein switches couple light, or the application of small-molecule drugs, to hyperpolarization or depolarization of the neuronal membrane or to changes in synaptic transmission. Inducible silencing, for example using small-molecule or light-gated channels, can be used to determine whether activity in specific cell types is necessary for behaviour. Inducible activation can be used to test whether activity in specific cell types is sufficient to drive behaviour. The light-sensitive channel channelrhodopsin-2 (ChR2) from *Chlamydomonas reinhardtii* has proven to be especially useful and versatile<sup>26</sup>: ChR2-expressing neurons can be entrained by light to fire precisely timed trains of action potentials<sup>27,28</sup>. ChR2 can thus establish causality between precise patterns of activity in a specific set of neurons and behaviour<sup>29–31</sup>. ChR2 can also be used to map the postsynaptic partners of ChR2-expressing neurons<sup>32</sup> and to identify specific types of neuron, tagged by ChR2 expression, in blind extracellular recordings<sup>33</sup>.

Protein sensors and switches can be targeted to specific cell types using a variety of genetic methods<sup>3</sup>. Genetic targeting is not limited to genetic model systems. For example, in some cases cell-type-specific expression can be achieved by including *cis*-regulatory elements in viral promoters<sup>34,35</sup>. However, by far the most versatile methods rely on transgenic mice expressing ‘drivers’ (typically Cre recombinase) in specific cell types<sup>3</sup> (Fig. 1). The driver mice can be mated with mice containing ‘floxed-stop’ reporter alleles to achieve expression of sensors or switches in the cell types of interest. Alternatively, the reporter can be carried in a Cre-dependent viral vector<sup>36</sup>. Cre mice are currently being produced in large-scale projects (for example the Gene Expression Nervous System Atlas (<http://www.gensat.org>) and the Allen Institute for Brain Science transgenic mouse study (<http://transgenicmouse.alleninstitute.org>)), informed by comprehensive gene expression databases (such as the Allen Mouse Brain Atlas (<http://mouse.brain-map.org>)).

Protein switches have already been used to analyse the contributions of specific cell types to behaviour. Early *in vivo* studies using molecular

Ntsr1-Cre line<sup>2</sup> crossed with reporter mice, labelling corticothalamic L6 neurons (**d**); Etv1-Cre line<sup>2</sup> crossed with reporter mice, labelling mainly L5a corticostriatal neurons (**e**); Glt25d2 line expressing green fluorescent protein, labelling mainly L5b neurons (**f**); spatially restricted Cre-dependent expression of fluorescent protein in L5a after injection of reporter virus<sup>36</sup> into Etv1-Cre mice (**g**). (Images courtesy of H. Zeng (Allen Institute for Brain Science, Seattle, Washington) (**b, c**), N. Heintz and E. Schmidt (GENSAT Project, Rockefeller University, New York) (**d–f**) and T. Mao (Janelia Farm Research Campus, Ashburn, Virginia) (**g**).

silencers were mainly designed to demonstrate the methods<sup>3</sup>. However, molecular silencers have begun to reveal the functions of specific cell types in the context of innate behaviour. For example, silencing somatostatin-positive neurons in the pre-Bötzinger complex causes rapid respiratory failure, linking activity in these neurons to the control of breathing<sup>35</sup>.

Photostimulation of ChR2-positive neurons *in vivo* has established causal links between electrical activity in specific types of neuron and behaviour. For example, dopaminergic neurons in the ventral tegmental area are thought to encode reward-prediction errors, but it is unclear whether changes in activity in these neurons are sufficient to signal reward or aversive stimuli. Expression of ChR2 in dopaminergic neurons in the ventral tegmental area allows control of the activity of these neurons in behaving mice<sup>31</sup>. Pairing short epochs of high-frequency photostimulation with mouse exploration of one of two chambers produced a place preference for that chamber, demonstrating that phasic firing in dopaminergic neurons by itself can cause behavioural conditioning.

So far, protein switches have been applied to manipulate relatively slow processes, in which behavioural changes are observed seconds or more after stimulation, driven by peptidergic<sup>30</sup> or neuromodulatory<sup>31</sup> systems. Typical reaction times for complex sensory-guided behaviours are on the order of 200 ms (ref. 37). The neural activity underlying these behaviours crosses multiple synapses. The core neural circuits implementing behaviour are likely to be driven largely by glutamatergic and GABAergic ( $\gamma$ -aminobutyric-acid-producing) neurons with fast dynamics in the 1–10-ms range. To reverse engineer these fast processes, it is therefore necessary to monitor behavioural parameters while measuring and controlling neural activity, ideally in a rapid closed loop, all on a millisecond-by-millisecond basis.

### Mouse behaviour

New molecular tools for cell-type-specific neurophysiology and manipulation facilitate genetic dissection of neural circuits in the mouse, but the limited demonstrated behavioural potential of the mouse is often perceived as a major obstacle. New mouse lines are routinely subjected to a battery of tests for the basic characterization of sensory, cognitive, motor and temperamental traits<sup>38</sup> (see also the Mouse Phenome Database from The Jackson Laboratory (<http://www.jax.org/phenome>)). These assays are used to describe differences across groups of animals. Although the standardization achieved using these tests is unmatched in the field of animal behaviour, the levels of behavioural control are often inadequate to determine the relationships between sensory stimuli, behaviour and neural activity.

Tasks based on mazes or arenas, such as the Morris water maze<sup>39</sup>, in which a mouse must remember and swim to the location of a hidden

platform, have shed light on the neural and molecular basis of spatial learning and memory<sup>40</sup>. Maze tasks have been adapted to assay sensory discrimination<sup>41</sup> and working memory<sup>42</sup>. However, maze tasks yield few trials (typically tens of trials) and stimulus control is relatively poor because the animal is free to move with respect to the stimuli.

The head-fixed, behaving monkey preparation has been the ‘gold standard’ in the exploration of the dynamics of the mammalian brain, and of the cerebral cortex in particular. Monkeys are typically trained on tasks designed to isolate the core sensory<sup>12,20</sup>, cognitive<sup>11</sup> or motor functions<sup>10</sup> of interest. Head fixation and body restraint provide a high degree of stimulus control (for example visual scene) and behavioural read-out (for example eye or arm position)<sup>10,12</sup>. Monkeys perform many hundreds of trials per day for a juice reward. The combination of precise stimulus control, monitoring of motor output and single-unit recordings over large numbers of trials is the foundation on which many conceptually rich and quantitative studies of the neural basis of sensation, cognition and movement have been built. Monkey experiments have also led the way in separating causation from correlation in neurophysiological experiments<sup>19,20</sup>, but cell-type-specific measurements and manipulation will probably remain exceptional in monkeys<sup>10</sup>. In contrast, in the mouse brain, cell-type-specific neurobiology is becoming routine.

Inspired by the experiments on behaving monkeys, procedures for quantitative head-fixed behaviours are being developed for mice. Motor learning in the vestibulo-ocular reflex, long studied in monkeys, has been adapted to the mouse<sup>43</sup>. Head fixation is central to this scheme, because precise control of head motion with respect to visual stimuli is essential, as is precise measurement of eye position. Head-fixed mice can also run freely on spherical treadmills<sup>44</sup>. In this experimental situation, complex terrains could be simulated in closed-loop virtual-reality environments, making possible the study of navigation with precise experimental control.

Beyond reflexive behaviour, mice have also been trained in choice-based, learned tasks. Studies in olfaction have come close to matching the standard of monkey experiments for experimental control and trial repeatability. In olfactory discrimination tasks, mice sample odours and learn to choose a correct response on the basis of the identities of two odours, often with performance levels approaching 100% for simple discriminations<sup>45,46</sup>. In these types of experiment, mice are motivated by thirst — as in most monkey studies but in contrast to most other rodent work — and routinely perform hundreds of trials.

In the remainder of this Review, we draw examples from the somatosensory system of mice. Rodents use their whiskers to detect and locate objects when moving through an environment<sup>47</sup>. The measurement of the locations of object features is also an important aspect of object identification. Inspired by previous work in freely moving rats<sup>48,49</sup>, we have trained head-fixed mice to locate an object (a vertical pole) near their heads with their whiskers (Fig. 3). In each trial, mice report with go/no-go lick responses whether the pole has been presented in a target

location or in a distractor location. Mice perform at high levels (>90% correct for simple discriminations) for hundreds of trials (Fig. 3c). High-speed imaging of whisker position reveals the whisker motor programs underlying somatosensory discrimination. Changes in whisker shape, caused by contact between whisker and object, report the mechanical inputs to the somatosensory system<sup>50</sup> (Fig. 3a). The object-localization task is ideally suited to probing the cortical basis of tactile spatial perception and sensorimotor integration in the context of active sensation.

Repeatability over hundreds of trials, a major strength of monkey studies that is now achievable with mice, allows powerful statistical analyses but also merits a caveat. In these tasks, animals become expert performers in a specific task. The neural activity, and even the basic circuits, underlying a specific behaviour can change profoundly with expertise<sup>51</sup>, and may differ from those underlying natural behaviours. Massively parallel recordings of single neurons may eventually obviate the statistical need to accumulate data over many repeated trials<sup>52</sup>.

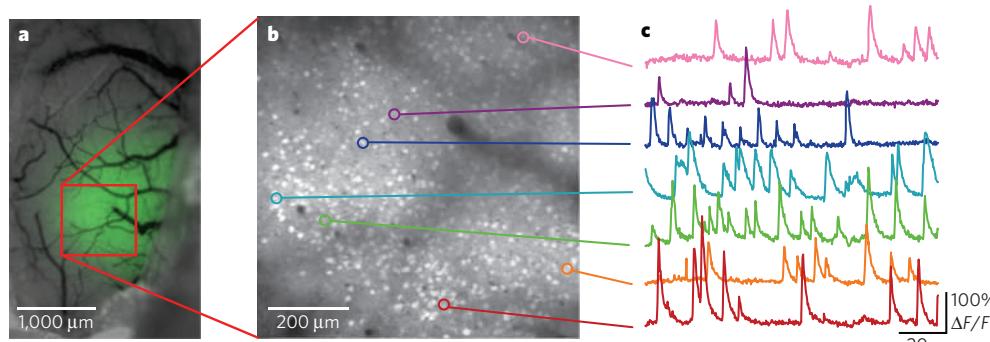
Behaving mice are emerging as an important model in systems neurobiology. Because of genetic access to cell types, the use of mice will allow more precise and reductionist neuron-level experiments than will the use of head-fixed monkeys. Next, we illustrate this point by outlining possible experiments in mice performing the somatosensory discrimination task.

### Reverse engineering neocortical circuits

Rodents move their whiskers in the horizontal plane to localize objects along the anterior–posterior axis (Fig. 3a). It is not clear what neural mechanisms underlie the encoding of horizontal object location. Object location is computed, at least in part, in the barrel cortex. Information about contact between whisker and object appears in the barrel cortex as a brief burst of action potentials coincident with mechanical forces on the whiskers<sup>53</sup> (Fig. 3b, c). As a whisker protracts, objects that are more distant are contacted later in a whisk cycle. The timing of these action potentials within the whisk cycle could therefore encode horizontal location<sup>47</sup>.

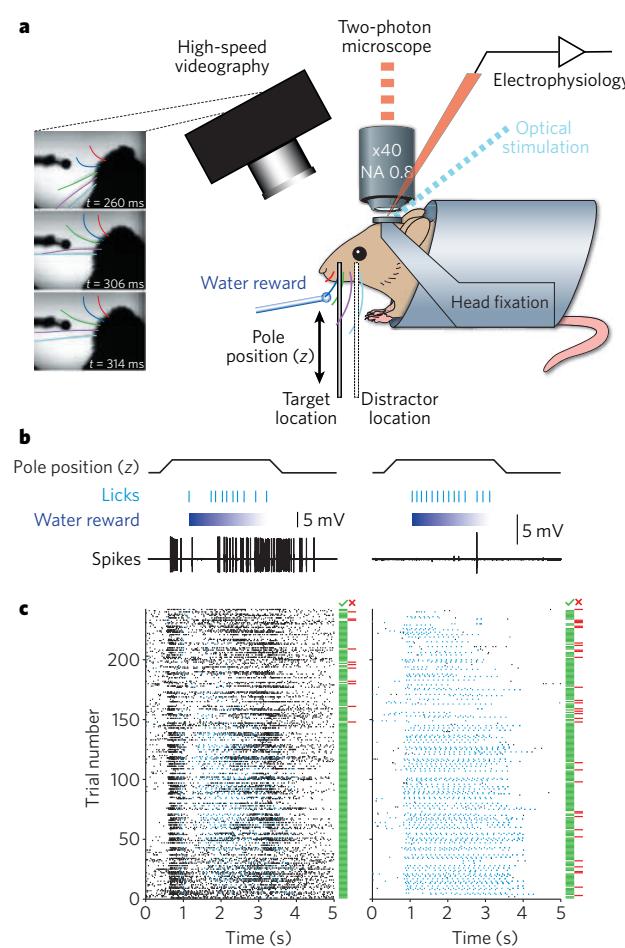
Information about whisker-object contact is conveyed by the ventral posterior medial nucleus of the thalamus to stellate cells in L4 barrels (Fig. 1a; L, cortical layer). The only other major excitatory input received by stellate cells comes from other stellate cells in the same barrel, which are presumably excited by deflection of the same whisker<sup>5</sup>. The L4 barrel relays peripheral contact signals to cortical targets, mainly in L3. L3 neurons project to L5 neurons, which include the major output neurons of the cortex.

To determine whether a short burst of activity (Fig. 3b, c) in L4 neurons is necessary and sufficient to perceive object location, molecular switches can be targeted to L4 neurons by using mouse lines expressing Cre in L4 (for example the Six3Cre transgenic mouse line 69 (ref. 56) or the Scnn1a-Tg3-Cre line from the Allen Institute for Brain Science; Fig. 1b). Stereotactic injection of adeno-associated virus, expressing switches in a



**Figure 2 | Monitoring the activity of large neuronal populations with a genetically encoded calcium indicator.** **a**, Overlay of bright-field (grey) and wide-field fluorescence (green, virally expressed indicator) images of mouse neocortex. **b**, Two-photon image showing approximately 500 L2/3 neurons

expressing the calcium indicator. **c**, Example traces showing neural-activity-induced calcium transients in multiple individual neurons. Images are from awake, head-fixed mice (see also Fig. 3).  $\Delta F/F$ , change in fluorescence divided by baseline fluorescence.



**Figure 3 | Tactile object localization in the head-fixed mouse.** **a**, Schematic of apparatus for behaviour, high-speed videography, neurophysiological recording, neuron-level imaging and optical stimulation. A head-fixed mouse moves its whiskers to determine the location of a small pole presented on one side of its head. In each trial, the pole is moved vertically to within reach of the whiskers, in either a target or a distractor location. Behavioural choice is reported with go/no-go licking. High-speed video measures the positions and shapes of whiskers as they search for and contact the pole (left-hand image panels). Neural activity can be monitored using electrophysiology and two-photon microscopy, or manipulated with optical stimulation. NA, numerical aperture. **b**, Example trials showing behaviour and action potentials for two neurons (left and right). **c**, Rasters of 242 trials for two neurons: black tick-marks represent action-potential times; blue tick-marks represent licks. Horizontal green and red bars to the right of each raster indicate trials that were correct and incorrect, respectively. Although recorded in the barrel cortex of the same mouse, the two cells show different patterns of activity.

Cre-dependent manner, causes expression in L4 neurons in these mice<sup>55</sup>. Somatotopic specificity can be achieved by injecting the virus into the barrel column corresponding to a specific whisker (Fig. 1g), aided by intrinsic signal imaging<sup>5</sup>.

Expression of small-molecule-activated K<sup>+</sup> channels<sup>57</sup> could be used to silence L4 neurons reversibly on a timescale of one hour, corresponding to a typical behavioural session. The behavioural performance in sessions before, during and after silencing would indicate whether activity in L4 neurons is necessary for object localization. However, prolonged silencing over many trials might confuse or discourage the animal because of loss of reward. This confound can probably be solved with rapid light-activated silencing methods, such as using the light-sensitive chloride pump *Natromonas pharaonis* halorhodopsin<sup>58,59</sup>, which can provide silencing at the level of single trials.

The timing of a brief burst of activity beginning in L4 neurons may be sufficient to determine horizontal object location. Precisely timed

ChR2 photostimulation of L4 neurons, with the goal of producing fictive objects, could be used to test this hypothesis. The speed of active sensation presents challenges: whiskers move millimetres in milliseconds. The photostimuli therefore need to be triggered with millisecond accuracy by whisker position. This type of closed-loop experiment is allowed by the precise behavioural control made possible by head fixation. If the mouse responds to photostimulation of L4 neurons (the fictive object) as if true contact had been made (based on the trained stimulus–response associations), it can be inferred that a timed burst of activity in L4 neurons is sufficient to lead to the percept of object contact at a particular location. Similar approaches could be used to impose more subtle perturbations of activity, such as changing the temporal correlations in spike trains.

Testing the necessity and sufficiency of different patterns of neural activity during tactile object localization can be extended to other genetically accessible cell types within the circuit (Fig. 1). For example, action-potential timing as a code for object distance can only be interpreted in the context of a signal encoding the position of the whisker over time<sup>47</sup>. Some barrel cortex neurons show activity that is modulated by whisking. A subset of neurons show brief responses modulated by the whisking cycle, suggesting that they could encode object location within whisker phase<sup>53</sup>. Cortical (whisker-related primary motor cortex) and thalamic (medial subdivision of the posterior nucleus) regions have been proposed as the source of the whisker-position reference signal<sup>47</sup> (Fig. 1a). Molecular switches, in the context of quantitative behaviour, are well suited to revealing the identity and properties of this reference signal.

### Synthetic brain science

In the previous section, we considered the manipulation of cell types to study the role of these neurons in normal sensation. However, stimulation of subsets of neurons can also be used as a ‘synthetic stimulus’ to probe fundamental properties of neural circuits.

For instance, the observation that some types of neuron show extremely sparse activity *in vivo*<sup>60</sup> raises the possibility that sparse activity patterns can be sufficient to drive behaviour. To test this possibility, ChR2 photostimulation has been used to mimic the sparse activity patterns observed in L2/3 pyramidal neurons<sup>61</sup> in awake, behaving mice<sup>29</sup>. Mice were trained to report photostimulation of ChR2-positive neurons by selecting one of two ports for a water reward. The number of activated neurons was quantified using histological and electrophysiological methods. After a learning period of a few weeks, mice were able to reliably report the activation of a small subset ( $\leq 1\%$ ) of L2/3 neurons; only 300 photostimulated action potentials were required to drive reliable behaviour<sup>29</sup>. This corresponds to a tiny fraction of the ongoing activity in the barrel cortex. Other experiments suggest that the stimulation even of individual neurons can have measurable effects on network activity<sup>62</sup> and behaviour<sup>22</sup>. These findings show that mechanisms exist to read out ultra-sparse activity from cortical networks.

Similar experiments could help unravel the circuit mechanisms underlying detection of ultra-sparse activity. One possibility is that the ChR2-positive neurons form part of a special cortical network defined by learning-induced plasticity at their output synapses. Alternatively, mice may generally learn to discern small increases in activity across the L2/3 population. These possibilities could be distinguished by training on photostimulation detection using one set of ChR2-positive neurons and testing with detection using another set of neurons. If, during learning, the ChR2-positive neurons do not form an ad hoc circuit, then mice should generalize to stimulation of a different set of intermingled neurons, with no change in threshold. The results of these experiments would profoundly affect our understanding of perceptual learning.

One striking observation in photostimulation-driven behaviour is that the number of stimulated neurons can be traded off against the number of action potentials per neuron<sup>29</sup>. In other words, trains of  $N$  action potentials (spread out over 250 ms) in  $n$  neurons are as efficacious in driving behaviour as one action potential in  $Nn$  neurons. This suggests that the networks reading out activity from L2/3 neurons integrate activity over times of at least 250 ms, the longest trains probed.

Future work may address the temporal limits, as well as the locus and mechanism, of this integration.

Detection of photostimulation could be used to study the essential neural components of decision making. The advantage over sensory-driven detection tasks is that the stimulus driving the decision, for example the ensemble of photostimulated L2/3 neurons, is under precise control. Even photostimulation close to the detection threshold is repeatable and not subject to the variability associated with sensory stimuli. Under these conditions of precise activation, it might be possible to track the evolution of network activity leading from deterministic stimuli to stochastic choice behaviour<sup>63</sup>.

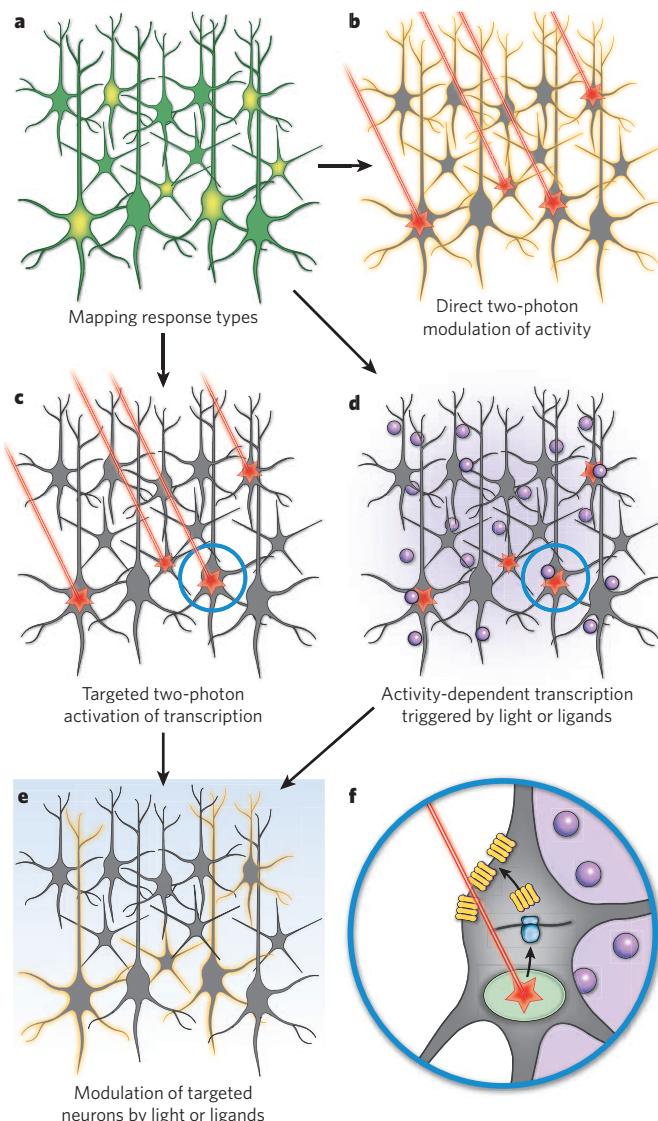
### Beyond cell types

So far we have labelled neurons as members of a cell type; but cell type is only a first approximation of the functional description of any one neuron. Although the activity patterns of neurons belonging to a particular cell type are distinct on average, neural responses are often extremely heterogeneous even for a cell type and brain region. For example, neighbouring L2/3 neurons in the rodent visual cortex can have sharp but divergent orientation tuning<sup>64</sup>. Similarly, neurons in the barrel cortex have diverse response types, including excitation by contact with an object, or tonic excitation coupled to whisking, as well as inhibition<sup>55,65</sup> (Fig. 3c). Neurons differ not only in their selectivity for particular stimuli (their ‘tuning’), but also in the amount of information they carry. The activity of some neurons bears no obvious relation to the task, whereas other neurons discriminate stimuli as well as the animal does<sup>66,67</sup> (Fig. 3c). Furthermore, the relationship between individual neurons and behaviour is flexible: experiments involving biofeedback have shown that the activity of individual motor cortex neurons is under volitional control and can be tuned to maximize reward<sup>68</sup>. Without manipulating the activity of neurons showing specific response types, however, there can be no definitive answer to the question of whether some neurons are more relevant to behaviour than others.

Although studies that directly target neuronal response types for manipulation have not yet been done, the technical challenges facing such experiments are rapidly being overcome. Functional imaging may allow the mapping of response types of tens of thousands of neurons in volumes on the order of a cubic millimetre<sup>64</sup> (Fig. 2). Neurons could then be selected for manipulation on the basis of their response type (Fig. 4). For example, if the neurons express ChR2, targeted groups of neurons could be photostimulated by two-photon excitation of ChR2 (Fig. 4b). However, a conventional focused laser excites only a tiny patch of neuronal membrane, containing a small amount of low-conductance ChR2, which produces insufficient depolarization to drive action potentials in most neurons<sup>69</sup>. Achieving routine two-photon stimulation of single neurons may require new types of ChR2 with longer open times<sup>70</sup> or greater conductances, combined with beam shaping to allow larger membrane areas to be excited<sup>69,71</sup>. In behaving animals it would then be possible to study how activity in subsets of neurons, selected on the basis of response type, contributes to behaviour.

A different strategy for targeting neurons on the basis of response type might rely on transcription activated by light<sup>72</sup> (Fig. 4c, f), or the conjunction of neural activity and an exogenous trigger (using small molecules or light) (Fig. 4d, f). In fact, existing transgenic mice couple neural activity to expression of protein markers<sup>73</sup>; molecular switches could be expressed instead, to manipulate the activity of neurons that were active in an earlier time window.

It may be possible to trigger expression of protein activators and silencers in arbitrary groups of neurons *in vivo*, using illumination of multiple individual neurons. The targeted neurons could be those exhibiting particular response types identified in prior imaging experiments. Single-cell resolution could be achieved by two-photon excitation (Fig. 4c, f). Subsequently, neurons expressing the switch could be activated or silenced using small molecules or diffuse light (Fig. 4e). This strategy, based on two-photon excitation of transcription followed by one-photon photostimulation of ChR2, might be less demanding of photophysics than direct two-photon photostimulation of ChR2.



**Figure 4 | Manipulating groups of neurons on the basis of their response type.** **a**, Mapping response types using optical indicators or electrodes. **b**, Modulation of activity by two-photon excitation of photoswitches (yellow) in neurons showing particular response types. **c, d, f**, Expression of genetically encoded switches by hypothetical transcription systems triggered directly by light (**c; f, left**) or by a combination of neuronal activity and small molecules (purple circles) or light (purple glow) (**d; f, right**). The cellular production and transport of protein switches (yellow) is indicated schematically (blue, ribosome). **e**, Manipulating neurons previously targeted for expression of a molecular switch (yellow). Activation (or inhibition) could be achieved using diffuse light or a small-molecule ligand (blue glow).

In general, the relationship between cell type and response type is not understood. Whereas cell type is a category defined largely by development, a neuron’s response type is most probably malleable, possibly even changing with behavioural context<sup>68</sup>. The anatomical underpinnings of the diverse neuronal response types are not known. The axons of excitatory neurons connect to only a small fraction of available target neurons of any given type. This sparse connectivity is known not to be random; rather, relatively strongly connected and interdigitated subnetworks are embedded within the cortical circuit defined by cell types<sup>8,9</sup>. It is likely that this anatomical fine-scale specificity in some way underlies different response types.

However, the question of which circuits determine any particular response type remains unanswered, even in the well-studied example

of orientation selectivity in the visual cortex<sup>17,64</sup>. A minimal explanation will require characterization of the inputs that drive a particular response type. One approach might involve measurement of population responses using [Ca<sup>2+</sup>] imaging and post hoc large-volume ultrastructural analysis to recover the detailed connectivity between characterized neurons<sup>74</sup>. Another approach could be based on labelling the target neuron and all of its inputs with a trans-synaptic virus<sup>75</sup>, for subsequent physiological analysis.

In this Review, we have advocated a programme of reverse engineering with the goal of linking patterns of activity in specific cell types to behaviour. This experimental programme would be most effective if it were tightly integrated with theory. Useful theoretical models could take many shapes. For example, semi-realistic compartmental models of single neurons, combined with information on the distributions of specific types of input within their dendritic arborizations, might reveal the mechanisms of how synaptic input interacts with postsynaptic membrane properties to drive particular response types. Other types of model might focus on the dynamics of the neural network or on information transmission within the network. The vast amounts of data collected in behavioural and physiological experiments can only be exploited with new approaches for data reduction and mining. Ultimately, progress in systems neuroscience will require the development of new theoretical tools in conjunction with cell-type-specific recordings and manipulations in behaving animals.

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