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More than a feeling: sensation from cortical stimulation

Kristina J Nielsen and Edward M Callaway

The authors are in the Systems Neurobiology Laboratories, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, California 92037, USA. e-mail: callaway@salk.edu

Abstract

Changes in neuronal firing underlie sensation, but how many neurons are needed to perceive these activity shifts? Two new studies in *Nature* suggest that the experimental modulation of only a few neurons can influence perception.

We detect environmental stimuli by perceiving the activity modulation in some subset of the 21 billion neurons in our brains¹, and the quality of this sensation depends on which neurons change their activity. But how many neurons are required for perception? Are particular types of neurons more effective than others in communicating these activity changes? Two papers recently published in *Nature* shed light on these issues by using different approaches to manipulate the activities of a few selected neurons in the somatosensory cortex of awake, behaving rodents^{2,3}. Their results indicate that rodents can detect changes in the activities of a very small number of cortical neurons, or even of an individual neuron. These studies point the way toward future experiments using increasingly sophisticated methods to tease apart the contributions of specific neural elements to behavior and perception.

In the first study, Houweling and Brecht² trained rats using cortical microstimulation, a technique in which neural activity is manipulated by passing electrical current into the brain. Rats were placed in front of a port that could be activated by licking responses and were rewarded with a small drink of water when they successfully licked immediately after stimulation of the somatosensory cortex. Stimulation trials were interleaved with catch trials, in which there was no stimulation. Incorrect licking during these trials was punished by imposing an additional delay before the start of the next trial. By imposing only minimal punishment for incorrect responses, this reward scheme was designed to bias animals toward a strategy of guessing in favor of stimulation when they were uncertain.

The authors initially used strong cortical microstimulation, and then gradually reduced the stimulus intensity until animals could reliably report the presence of a weak stimulating current. These well-trained animals were then tested for their ability to respond to the stimulation of a single cortical neuron. To stimulate a single neuron, the authors used a ‘juxtacellular’ stimulation protocol⁴, positioning the tip of a glass micropipette against a neuronal cell body (Fig. 1a), to generate about 15 action potentials. Stimulation was limited to neurons in cortical layers 4 and 5, but included both excitatory and inhibitory neurons, as identified by their spike shapes and firing rates.

Juxtacellular stimulation biased animals to report stimulation (through licking) more often than they did in catch trials lacking stimulation. These effects were small, more variable than with microstimulation, and only became significant when data were pooled over all tested neurons. During catch trials with no stimulation, animals ‘guessed’ that they should lick 18% of the time. With juxtacellular stimulation, the rate of licking rose to 22%. Because the response rate in stimulation trials was significantly larger than the false-positive rate in nonstimulation trials, these results indicate that, at least in some cases, stimulation of just a single neuron in somatosensory barrel cortex can bias behavior.

Huber *et al.*³ used a different approach to stimulate selected neurons in the mouse barrel cortex. They selectively introduced the light-gated algal channel channelrhodopsin-2 (ChR2)⁵ into layer 2/3 excitatory neurons across a wide expanse of one cortical hemisphere. This allowed for the stimulation of ChR2-expressing neurons in response to blue-light exposure (Fig. 1b). Before testing the influence of stimulation on behavior, the authors first demonstrated that shining pulses of blue light onto the exposed surface of the somatosensory barrel cortex did indeed trigger neurons to fire action potentials. Neurons fired in synchrony with brief (1 ms) light pulses, and could be driven to respond with firing rates as high as 50 Hz. The authors also demonstrated that changes in the intensity of the blue light influenced the size of the activated neuronal population. The more intense the light pulses, the larger the number of neurons stimulated.

Similar to the rats in the study of Houweling and Brecht², the mice in this study³ were trained to report stimulation, but with a slightly different task. Mice initiated each trial by sticking their nose into a central port. Subsequently, photostimulation was or was not delivered to the barrel cortex. Animals were trained to report stimulation by placing their nose into a port on one side of the center initiation port and to report no stimulation by placing their nose into a port on the opposite side of the center port. Correct responses, correctly classifying a trial as stimulation or no stimulation, were rewarded with a drop of water.

After a few training sessions, the mice reliably reported photostimulation with an average of 72–93% correct responses. During the training sessions, the authors used high-intensity light, triggering neurons to generate five action potentials. Subsequent experiments tested how many neurons needed to be activated, and how many action potentials needed to be generated, to allow for reliable performance. In general, performance decreased when fewer neurons were activated and fewer action potentials were generated. However, as long as enough neurons were stimulated, the mice could reliably report even single action potentials triggered in these neurons. Overall, the activity of about 60 neurons was sufficient to drive reliable performance (more than 65% correct choices) if these neurons generated five action potentials. If neurons only fired a single action potential during stimulation, about 300 neurons had to be stimulated to achieve the same performance levels.

Microstimulation was pioneered more than 100 years ago by Fritsch and Hitzig, who studied the consequences of stimulating different brain regions in dogs⁶. Since then, this technique has been used to probe a variety of complex functions, ranging from the execution of movements^{7–9} to perception¹⁰ and attention¹¹. Although these and other experiments clearly demonstrate that small populations of cortical neurons can bias perception and behavior, they have not allowed for the assessment of the minimal numbers of neurons or neuron subtypes that are sufficient to drive this influence. Both of the new papers^{2,3} provide insights into minimal stimulation perceptual thresholds (Fig. 1c) and demonstrate methods that should allow us to probe the roles of additional cell types. Juxtacellular stimulation allows for the controlled stimulation of a single cell, allowing one to probe specific types of excitatory or inhibitory neurons individually. Although the data are limited, Houweling and Brecht's results² indicate that stimulation of excitatory cells and interneurons may have different consequences for behavior. Stimulation of the putative interneurons led to the largest behavioral effects observed in this study, but effects were also more variable. Follow-up studies should investigate further the relationships between the stimulated cell type and the nature and strength of perceptual or behavioral influences.

The paper by Huber *et al.*³ provides a different approach to cell type-specific stimulation. In their study, expression of ChR2 was limited to excitatory neurons in layer 2/3 by introducing the DNA encoding ChR2 via *in utero* electroporation into the progenitors of these cells. Thus, photostimulation was limited to a subgroup of similar neurons, demonstrating that selective

stimulation of this cell type is sufficient to generate a behavioral bias. Future studies using this method can take advantage of genetically targeted strategies to restrict ChR2 expression to other cell types¹². This would allow researchers to examine the influence on perception and behavior of numerous other distinct cortical elements.

There is little doubt that the effects observed in these studies are also related to changes in the activity of additional neurons downstream of the initial stimulation event. It would therefore be highly desirable to selectively stimulate one neuronal population while monitoring the activity of other neurons (for example, as in ref. ¹³) to infer which downstream targets might also be involved in these behavioral manifestations. Furthermore, the combination of targeted stimulation of some neurons with the selective inactivation of downstream neurons, perhaps using the light-gated chloride pump triggered by longer wavelength light^{14,15}, might provide insight into which neurons are necessary, as well as sufficient, for the generation of behavioral biases.

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Figure 1.

Stimulation protocols used in the new studies. Both groups stimulated neurons in the rodent somatosensory barrel cortex. **(a)** In the juxtacellular stimulation experiment of Houweling and Brecht², a single neuron was stimulated by closely approaching its cell body with a glass pipette and passing a current into the neuron. These experiments preferentially targeted neurons in deeper cortical layers 4, 5 and 6. **(b)** In contrast, Huber *et al.*³ manipulated cells to express the light-gated channel ChR2, allowing for the stimulation of ChR2-expressing neurons with blue light. Expression of ChR2 was restricted to excitatory neurons in cortical layer 2/3. **(c)** Both groups trained rodents to report stimulation of neurons in somatosensory barrel cortex.