Probing neural circuitry and function with electrical microstimulation

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Review

Probing neural circuitry and function with electrical microstimulation

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Since the discovery of the nervous system's electrical excitability more than 200 years ago, neuroscientists have used electrical stimulation to manipulate brain activity in order to study its function. Microstimulation has been a valuable technique for probing neural circuitry and identifying networks of neurons that underlie perception, movement and cognition. In this review, we focus on the use of stimulation in behaving primates, an experimental system that permits causal inferences to be made about the effect of stimulation-induced activity on the resulting behaviour or neural signals elsewhere in the brain.

Keywords: electrical stimulation; monkey; behaviour

1. INTRODUCTION

(a) *A little history*

The 1870 discovery by Fritsch & Hitzig that electrical stimulation applied to the surface of cerebral cortex evokes discrete, reproducible movements is a landmark in the history of neuroscience. Upon applying electrical stimulation to the surface of the cortex of dogs, Fritsch & Hitzig observed largely contralateral movements of the body. The movements, evoked with brief pulses of direct current, tended to involve different muscle groups when the stimulation was applied to different regions of cortex. This provided the first evidence that specific functions could be localized within the brain [1]. The localization of specific functions to particular regions and circuits of the brain remains a fundamental goal of neuroscience, and electrical stimulation has been an essential tool for causally relating neural circuits to sensation, movement and cognition.

(b) *Activation of neural tissue with electrical microstimulation*

The technique of electrical stimulation has been refined considerably since the time of Fritsch and Hitzig, who applied a pulse of direct current to electrodes on the surface of the brain via a battery [2]. Microstimulation protocols have been developed that produce reliable activation and minimize damage to neural tissue [3]. The parameters affecting the magnitude and extent of neural activity induced by the application of electrical current have been reviewed previously [4,5]. Two main factors determine the spatial extent of neural activation by microstimulation: the physical spread of current, with the current strength falling off proportional to the

square of the distance from the electrode tip, and the excitability of the neural elements within that volume, where myelinated axons and the initial segment are most excitable [5]. This topic remains an active area of research [6,7] (see the electronic supplementary material for a detailed discussion of how microstimulation affects neural activity). Several experimenter-controlled parameters alter the efficacy of microstimulation, including pulse duration and polarity (i.e. cathodal versus anodal), inter-pulse interval/frequency, current amplitude and the temporal characteristics of the current pulse-train. Most experiments use biphasic pulses composed of equal duration cathodal and anodal phases so that there is little or no net charge delivered to the cortex, thereby reducing the electrolytic damage to brain tissue [3]. Frequency is typically 30–333 Hz, with current amplitudes from 5–500 µA. Pulses may be anodal or cathodal leading, with cathodal-leading being more effective as the initial pulse is depolarizing.

2. PERCEPTION

(a) *Evoking sensations with microstimulation*

Some of the earliest experiments to examine the influence of stimulation on perception came from physicians treating conscious human patients, an approach that offers a unique opportunity to 'speak to "the preparation" and have it answer you' [8]. Foerster ([9], in Brindley & Lewin [10]) found that when he stimulated points on the surface of the occipital cortex, patients reported seeing localized spots of light, known as phosphenes, at locations that varied depending upon where the stimulation was applied. Penfield's extensive studies of the effect of electrical stimulation throughout the brain included reports of localized 'crude visual sensation' in primary visual cortex that produced blindness in the affected region of space [8]. In an effort to develop a visual prosthesis, Brindley & Lewin [10] implanted an

array of electrodes on the surface of occipital cortex of a blind patient, allowing them to systematically examine the topographical map of visual space found in human occipital cortex based on the perceived location of stimulation-induced phosphenes.

While monkeys are unable to describe the visual percepts elicited via microstimulation, microstimulation of primary visual cortex (V1) in monkeys nevertheless produces experimental effects that are consistent with phosphene induction. Stimulation can evoke eye movements to the location matching the visual receptive field (RF) of cells at the stimulated site, interfere with other visual tasks, or be detected by animals trained to explicitly report the detection of microstimulation [11]. Murphy & Maunsell [12,13] trained monkeys to detect and report electrical stimulation in a variety of brain areas throughout the visual cortical hierarchy, from V1 to the frontal eye field (FEF) in prefrontal cortex. They found that the reliability of detection was always proportional to the microstimulation current, allowing measurement of detection thresholds at each stimulation site. Thresholds gradually increased from earlier to later visual areas. More recent work highlights the difference between the percept evoked via microstimulation and the experience associated with natural visual stimulation [14]. Monkeys trained to detect microstimulation of a particular V1 site can, with practice, significantly reduce their detection threshold at that site, but this is accompanied by a decreased sensitivity at detecting a veridical visual stimulus at the corresponding location. Subsequent retraining to original visual thresholds resets the microstimulation threshold to a high level. These findings suggest that plastic changes occur over the course of extensive training to optimize detection of either microstimulation or a visual stimulus to the detriment of the other type.

(b) *Modulating perception within a behavioural task*

Microstimulation has been widely used in monkeys performing behavioural tasks, where stimulation-driven changes in neural activity interact with task-related sensory stimulation. One classic example of this approach is the work of Newsome and colleagues, who tested whether changes in the activity of middle temporal (MT) neurons are sufficient to alter an animal's perception of visual motion using a rigorous psychophysical measure of perceptual performance [15]. They trained monkeys to discriminate the motion of dots moving within an aperture. The motion varied in the degree of directional coherence among the dots, from 100 per cent (all dots moving in the same direction) to 0 per cent (all dots moving randomly). As in V1, MT neurons are organized into columns in which nearby cells are selective for the same direction of motion [16], thus allowing the experimenter to stimulate similarly tuned neurons. Microstimulation of MT biased the monkeys' perception towards the preferred direction of neurons in the stimulated column, particularly when the motion stimulus was only weakly coherent. The consistent effect of stimulation on motion perception irrespective of the veridical visual stimulus suggests that stimulation-driven activity was combined with visually driven activity in an additive manner. A subsequent study suggested that

neural signals produced by microstimulation are filtered according to behavioural significance, just like the information that enters the visual system through the eye [17]. Similar experimental designs were used to demonstrate the role of MT in depth perception by DeAngelis *et al.*, and area MST in the perception of heading by Britten *et al.* (reviewed in [18]).

Afraz *et al.* [19] found that microstimulation of inferotemporal (IT) cortex influenced face perception. Neurons in IT respond selectively to complex visual stimuli, and some neurons respond selectively to faces [20]; these face-selective cells cluster in patches within IT [21,22]. Afraz *et al.* [19] trained monkeys to perform a face versus non-face categorization task, while viewing noisy images of faces or other objects. Stimulation of face-selective sites in IT cortex resulted in an increased tendency for monkeys to classify noisy stimuli as faces. The strength of this effect was correlated both with selectivity at the stimulated site and at the neighbouring sites: stimulation of sites with a stronger preference for faces and face-preferring neighbouring sites tended to have a greater effect on behaviour.

Microstimulation has also been used to probe the role of somatosensory cortex in tactile discrimination. Romo *et al.* [23] conducted experiments in the primary somatosensory cortex, where the frequency of certain neuronal responses correlates with the frequency of mechanical vibrations applied to the skin. Monkeys were trained to compare the frequencies of mechanical vibration applied to the fingertip during two discrete periods. After learning the task, one period of tactile stimulation was replaced with microstimulation of sites within the somatosensory cortex. Discrimination performance was the same regardless of whether monkeys made comparisons between two tactile stimuli, or between a tactile stimulus and microstimulation. Further experiments showed that monkeys continued to perform the task even when both periods of tactile stimulation were replaced with microstimulation of somatosensory cortex [24].

3. MOVEMENT
(a) *Evoking eye movements*
Conjugate eye movements, in which the two eyes move in the same direction, were among the movements Ferrier observed in his early stimulation experiments [25]. Nearly a century later, Robinson *et al.* precisely measured saccadic eye movements evoked by microstimulation of the FEF and of the superior colliculus (SC) [26,27]. The stimulation-evoked eye movements they observed resembled endogenous saccades, exhibiting the stereotypical relationship between saccade amplitude and duration characteristic of natural saccades [28]. Similarly, changes in gaze typically involve both eye and head movements, and microstimulation of the SC of head-unrestrained monkeys produces combined eye-and-head movements whose velocities, amplitudes and relative eye/head contributions mimic visually guided gaze shifts [29]. In addition to the FEF and the SC, eye movements can be evoked by microstimulation of the supplementary eye field [30], the lateral intraparietal area (LIP) [31], as well as other parietal and occipital areas [32], although higher stimulation currents are typically required.

The amplitude and direction of electrically evoked saccades vary systematically according to electrode position
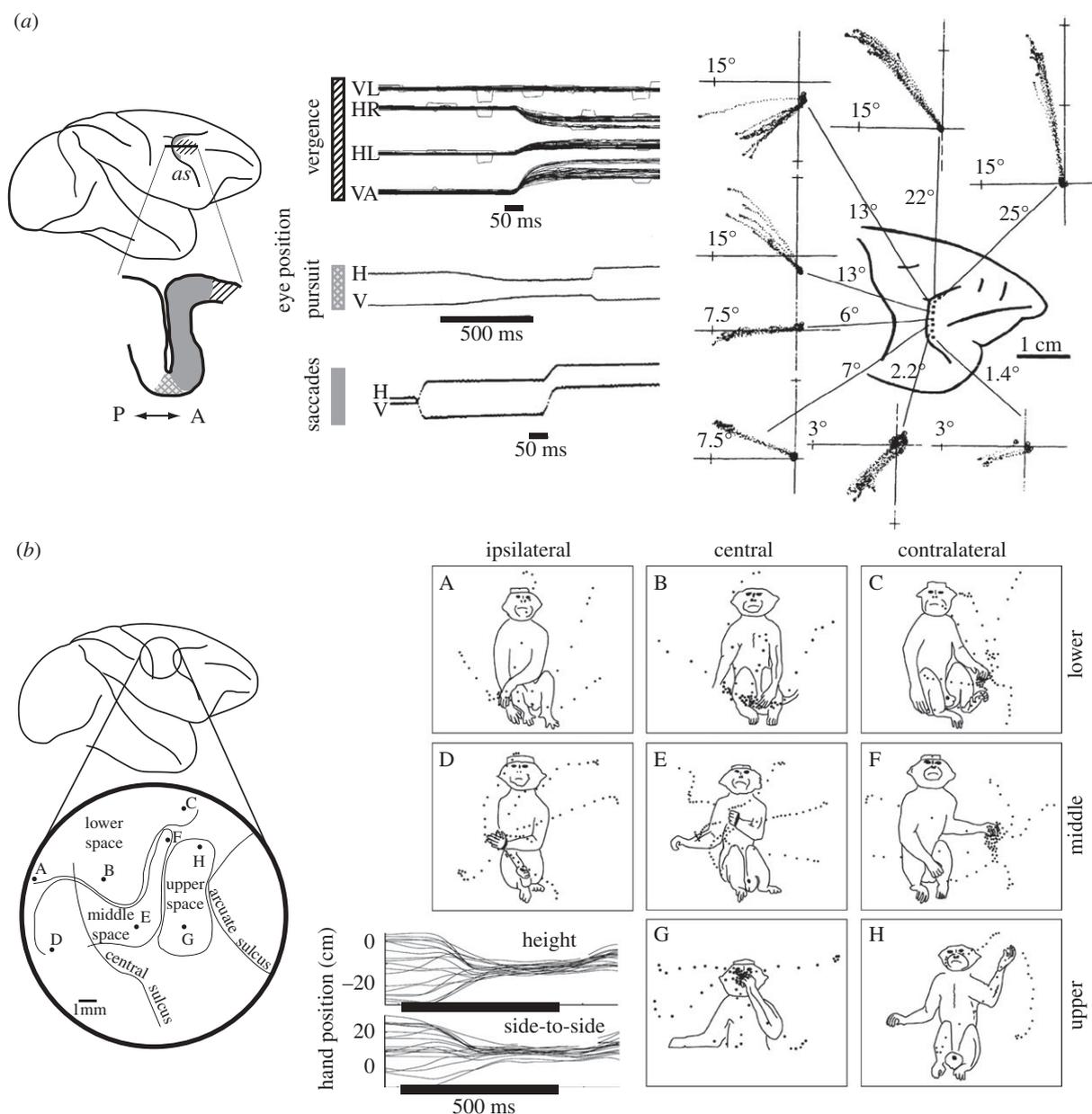
Figure 1. Evoking eye and skeletal movements with microstimulation. (*a*) Microstimulation of different regions inside and surrounding the arcuate sulcus (*as*) produces vergence (striped), smooth pursuit (plaid) and saccadic (solid) eye movements. Map of saccade amplitudes and directions evoked by microstimulation of the frontal eye field sites shown. (*b*) Microstimulation of motor and premotor for 500 ms evoked complex, coordinated movements terminating in a fixed final posture regardless of the initial position. Left: traces show hand position during microstimulation. (A–H): map of arm movements evoked by microstimulation of the motor and premotor sites shown. Dotted lines indicate path of hand movement, ending in the position shown. Abbreviations: P, posterior; A, anterior; VL, vertical left; HR, horizontal right; HL, horizontal left; VA, vergence angle; H, horizontal; V, vertical. Adapted from [34–36,50,51]

within the FEF, independent of starting eye position (figure 1*a*). Microstimulation parameters such as the frequency, train duration and current amplitude do not dramatically alter the evoked saccade vector, but rather the saccades are 'all-or-nothing', with changes in microstimulation parameters at a given site changing only the probability of evoking a saccade, but not its amplitude or direction. Robinson [26] obtained similar results in the SC. The endpoint of saccades evoked from the SC by electrical stimulation was shown to correspond closely to the spatial response field of single units near the electrode tip, which respond selectively to saccades of a particular amplitude and direction [33], and a similar correspondence was subsequently demonstrated in FEF [34].

In addition to examining the neural basis of saccadic eye movements, microstimulation has been used to study the neural basis of oculomotor vergence and pursuit (figure 1*a*). Microstimulation of a region lying just anterior to the FEF produces short-latency vergence movements [35]. Microstimulation of a sub-region of the FEF which lies buried in the fundus of the arcuate sulcus produces smooth eye movements [36], and appears to modify the gain of the visuomotor transmission used for pursuit [37].

(b) Pairing microstimulation with lesions or reversible inactivation
Pairing microstimulation of one area with lesions or pharmacological inactivation of another area can demonstrate the

sufficiency or necessity of interconnected areas contributing to a given task. In the oculomotor system, the FEF is known to project to brainstem oculomotor nuclei both directly and indirectly via the SC [38]. Lesions of the FEF alone produce minor deficits in saccade targeting and metrics [39], while inactivation of the SC produces slightly more robust defects [40]. Ablation of both areas produces profound deficits, permanently eliminating virtually all saccades [39]. Early studies combining ablation of either SC or FEF with microstimulation of the complimentary area [41] showed that electrically evoked saccades from one area were unaffected by ablation of the other. Recently however, Hanes & Wurtz [42] found that reversible inactivation of the SC significantly reduced the efficacy of FEF microstimulation. These results suggest that neither the FEF nor the SC is absolutely necessary for eye movements, though the FEF may normally send signals through the SC.

(c) Interaction of stimulation-evoked and endogenous saccades

Electrically evoked eye movements also interact with visually guided movements in much the same way that two voluntary saccades interact—for example, saccades compensate for prior eye movements in order to bring the eyes to a fixed location in space [43]. When a saccade is evoked by SC microstimulation immediately prior to the execution of a visually guided saccade, the subsequent (voluntary) saccade is nonetheless directed to the target location. In other words, the oculomotor system compensates for the electrically evoked saccade. The ability of the oculomotor system to compensate for the electrically evoked saccade displacement, in the absence of voluntary motor planning or visual feedback, provides evidence for the existence of a 'corollary discharge' signal carrying feedback about eye position during eye movements [44]. A second key finding of the study of Sparks & Mays [43] is that the precise endpoints of the microstimulation-evoked saccades are influenced by the ongoing eye movement preparation. This influence has since been used as an index of shifting attention [45], invisible target tracking [46] and evolving perceptual decisions [47].

(d) Evoking complex skeletal movements

Motor cortex was the first area to have its functional organization mapped by observing the effects of electrical stimulation [2,8,25], but how the pattern of activity among neurons in motor and premotor cortex (PM) gives rise to particular skeletal movements is still largely an unanswered question. Many studies have reported muscle twitches evoked by microstimulation of both primary motor cortex and PM (e.g. [48,49]). However, these studies used relatively short microstimulation trains (typically <50 ms). Graziano *et al.* investigated the effects of longer (500 ms) trains of microstimulation of motor and PM [50]. This timescale corresponds to both natural reaching movements and the neural responses observed in these brain areas during reaching tasks. Using these longer stimulation trains, microstimulation of the primary motor cortex and adjacent PM evoked complex, coordinated movements involving multiple joints to reach a fixed final posture. For example, in some evoked movements, the monkey would bring its hand towards its mouth with a closed, grip-like hand

posture and open its mouth. Microstimulation at different cortical sites yielded a variety of final arm postures, with final hand position converging on a specific location in space regardless of the initial posture (figure 1b).

Although the complex movements described by Graziano *et al.* [50] unfolded over the course of the several hundred millisecond stimulation train, they nonetheless resulted from short-latency differential activation of complimentary muscle groups. By combining motor-cortex microstimulation with electromyographic (EMG) recordings of muscle activation, the authors were able to measure EMG responses with very short latencies relative to stimulation onset [51]. For example, depending on the initial posture, either the tricep or bicep could contract within as little as approximately 7 ms of microstimulation onset to bring the arm to a final posture. As in natural movements, relative activation of antagonist muscles varied according to the initial joint position; velocity profiles for these movements were also similar to those of spontaneous natural movements. In addition, the arm postures evoked by microstimulation revealed an organization across the cortex in which the height of the final posture (head-to-toe) varied along the dorsoventral axis and the distance of the final posture from the midline varied along the rostrocaudal axis (figure 1b). Microstimulation of ventral PM or parietal area VIP evokes complex defensive postures including gaze centring, facial squint, head turn and raised arm with outward-turned hand [50,52].

(e) Disrupting motor planning

When the pattern of neural activity evoked by microstimulation differs sufficiently from that required to produce a particular movement, microstimulation may disrupt or delay voluntary movements rather than evoking movements. Although microstimulation of the FEF can evoke eye movements, sub-threshold microstimulation of the same site will delay execution of a voluntary saccade of a different vector [53]. A similar approach was used to study movement planning in motor cortex [54]. Psychophysical and physiological evidence supports the idea that voluntary movements are planned before they are executed (e.g. [55]). Neural correlates of this preparatory process have been observed in PM, which contains neurons that are active during a delay period and are selective for particular movements (e.g. [56]). Churchland & Shenoy tested whether this delay period activity has a causal role in reach-preparation by disrupting putative preparatory activity in dorsal PM (PMd) using microstimulation. Microstimulation of PMd during movement planning prior to a go cue slowed monkeys' reaction times (RT) when compared with control trials, while microstimulation of adjacent primary motor cortex did not affect reach RT. The authors concluded that a reach preparation process takes place in PMd during the delay period, and that movements will be delayed until this process is accurately completed.

4. COGNITION
(a) Driving attention and decision-making

In the past decade, many studies have examined the influence of microstimulation on behaviour in ways distinct from the effects of a purely sensory or motor bias.

Moore & Fallah [57] used microstimulation to test whether oculomotor commands play a role in covert attention. Psychophysical research demonstrates a close link between attention and eye movements (reviewed in [58]). Moore & Fallah drew upon these diverse findings and tested whether microstimulating neurons in the FEF, with currents that were too low to evoke a saccade, would nonetheless direct attention to the location represented at the stimulation site. Monkeys were trained to perform a covert attention task in which they had to detect a small change in luminance of a target in the presence of distracters. Sub-threshold microstimulation improved performance on the attention task [57]. Subsequent studies examining the relationship between eye movements and covert attention showed that improvements in attention could also be driven by sub-threshold microstimulation of other oculomotor regions, including the SC [59].

The apparent dual role of the FEF in controlling both saccades and covert attention raises the question of how the two processes interact during visually guided behaviour. Schafer & Moore [60] used a paradigm which pitted potential attentional effects of FEF stimulation against saccadic effects. When monkeys make voluntary saccades to a sinusoidal grating drifting within a stationary aperture, the average endpoint of their eye movements is slightly displaced in the direction of motion, consistent with an illusory shift in the perceived position of the grating (figure 2a). Schafer and Moore examined the effect of sub-threshold microstimulation on the endpoints of voluntary saccades to drifting gratings. Based on FEF's role as a motor structure, one can make a simple prediction for the effect of microstimulation: injection of a fixed vector signal should reduce the influence of the drifting grating on the saccade endpoints and bring the distribution of saccades closer to the central, evoked-saccade vector (figure 2b). However, based on the evidence that sub-threshold microstimulation of FEF drives spatial attention, a different result is suggested: attending to the grating should enhance perception of the motion producing a greater displacement of saccades along the direction of motion, away from the central vector (figure 2b). The authors found that when voluntary saccades were paired with sub-threshold microstimulation, the effect of the motion-induced displacement of saccade trajectories was enhanced, not decreased (figure 2c,d). Thus, the attentional effects of FEF perturbation effectively controlled the simultaneously planned saccades.

Microstimulation has also been used to study decision-making in the random dot motion task [61] used previously to study motion perception. One candidate area for the accumulation of evidence and decision-making in this task is the LIP. LIP neurons exhibit spatially specific visual and saccade-related activity, and during the random dot motion task LIP firing rates appear to climb to a threshold just before a saccade is executed to a target in their receptive field [62]. To causally examine the proposed role of LIP neurons in decision-making, Hanks and colleagues applied microstimulation to LIP sites with receptive fields that overlapped a target location during a reaction-time motion discrimination task [61]. Microstimulation of area LIP biased monkeys to choose the direction of motion associated with the
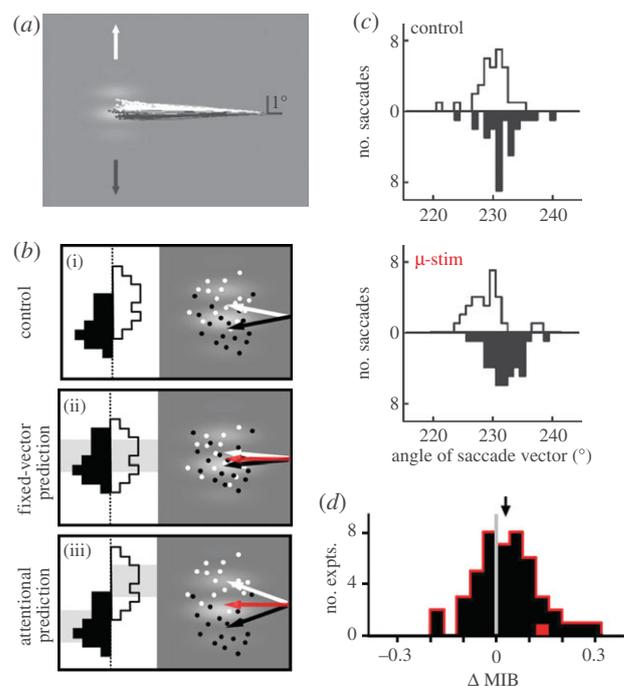

Figure 2. Effect of FEF microstimulation on the visual guidance of saccadic eye movements. (a) The motion-induced bias (MIB), in which the endpoints of saccades to a moving grating deviate slightly in the direction of grating motion, up (white arrow and eye traces) versus down (black arrow and eye traces). (b) Predicted purely motor or 'attentional' effects of FEF microstimulation on saccade endpoints. Histograms (i, control) show angle of deviation of saccade endpoints, arrows show average vector for upward (white) versus downward (black) grating motion. The fixed-vector hypothesis (ii) predicts injection of a fixed signal matching the evoked saccade vector (red arrow) regardless of the grating motion (grey shading on histogram), bringing mean upward and downward saccades closer together, reducing the MIB. The attentional hypothesis (iii) predicts an increasing influence of visual motion on saccade endpoints, increasing the MIB. (c) An example experiment showing the effect of microstimulation on the MIB. Histogram of saccade vector angles for (i) control ($n = 62$) and (ii) microstimulation ($n = 70$) trials. (d) Population analysis: the difference in the MIB between control and microstimulation. Microstimulation increased the MIB, consistent with the attentional hypothesis. Adapted from [60].

target that was placed in the RF of the LIP site, showing that LIP microstimulation can bias perceptual decision-making. Differential effects on choice probability and RT when microstimulating LIP versus MT in an identical task [63] were consistent with a model in which MT provides moment-by-moment sensory evidence to LIP, which integrates evidence in favour of motion in each direction until some threshold is reached and an eye movement is triggered.

(b) *Driving neural signatures of attention*

Moore & Armstrong [64] examined whether FEF microstimulation modulates visual responses elsewhere in cortex as spatial attention has been shown to do [65]. The FEF projects both to the SC and brainstem nuclei involved in executing eye movements, as well as to extrastriate visual cortex; these feedback projections provide a
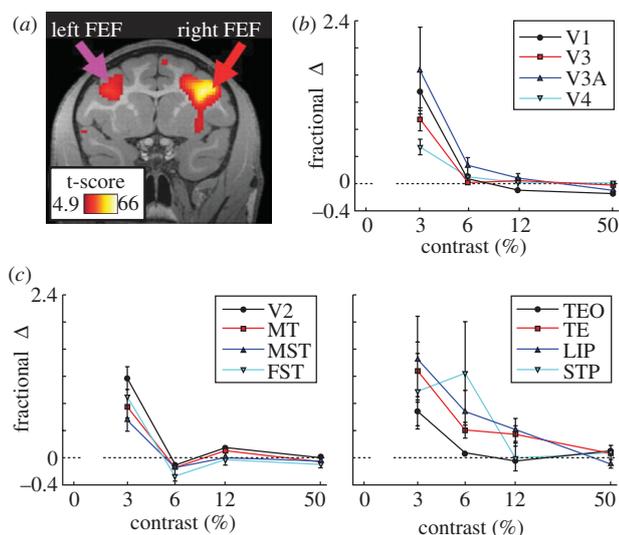
Figure 3. Effect of FEF microstimulation on BOLD activity in visual cortical areas of awake monkeys. (*a*) Differential BOLD activity for trials with and without right FEF microstimulation, showing activation of the stimulated and contralateral (left) FEF. (*b*,*c*) Difference (microstimulation versus control) in BOLD responses to stimuli of varying contrasts across areas of visual cortex (V1, V2, etc). FST, fundus of the superior temporal sulcus; STP; superior temporal polysensory area. Adapted from [67].

pathway by which the FEF might influence visual cortical responses. Moore and Armstrong applied microstimulation to the FEF at sub-threshold currents, like those shown to produce behavioural improvements, while recording from V4 neurons with RFs that overlapped the movement field of the stimulation site. V4 baseline firing and visual responses were recorded in the presence and absence of microstimulation. Although microstimulation did not increase the firing rate of V4 neurons in the absence of a visual stimulus, responses to both preferred and non-preferred stimuli were increased [64]. Further studies showed that the effects of FEF stimulation on V4 responses are strikingly similar to the effects observed during covert attention [66].

Ekstrom *et al.* [67] investigated the stimulation-induced enhancement of visual signals in several areas throughout the brain by combining microstimulation of the FEF with functional magnetic resonance imaging (fMRI) of awake monkeys (figure 3*a*). They measured blood oxygen level-dependent (BOLD) responses in visual cortex induced by FEF microstimulation, visual stimulation, or the combination of FEF microstimulation and visual stimulation at varying contrasts (figure 3*b*,*c*). Microstimulation interacted with visually driven responses in several areas. In addition, the impact of microstimulation on responses in V4 was stronger in the presence of visual distracters, and for visual stimuli of low contrast, consistent with electrophysiological studies [64,68].

(c) *Stimulation-induced learning and plasticity*

In the studies described thus far, microstimulation was used to influence behaviour or neural responses of the animal on a trial-by-trial basis. It is also possible to produce microstimulation effects which build up over time,

for example over the course of learning a sensory-motor association or seeking to directly induce plasticity between two sets of neurons. Williams & Eskandar [69] had monkeys learn by trial-and-error to associate images with one of four joystick movements. Many neurons in the anterior caudate are modulated by auditory reward feedback (a tone just prior to receiving juice) and reward (the juice itself). To look for a causal relationship between this caudate activity and learning rate, they applied microstimulation during the feedback and reward period of correct trials for only one of the images in a given block. They found that monkeys reached criterion more quickly for the image paired with microstimulation, though the final level of performance achieved was unchanged, thus demonstrating that anterior caudate activity can contribute to the formation of sensory-motor associations.

A large body of work has used microstimulation to examine the mechanisms of synaptic plasticity, especially the form known as long-term potentiation, both *in vitro* and *in vivo* [70]. Prolonged microstimulation has also been shown to induce cortical plasticity, for example expanding representations of particular body parts in somatosensory cortex [71]. Most of these stimulation protocols use fixed-frequency stimulation. One recent study by Jackson *et al.* [72] attempted to demonstrate similar plasticity effects *in vivo* with a more naturalistic stimulation pattern. Using a multi-electrode array implanted in motor cortex, Jackson *et al.* created an artificial connection between two sites by triggering microstimulation of one site immediately after recording an action potential at the other site. After the conditioning period, movements evoked from the recording site had become more similar to those evoked originally from the stimulation site, while control sites not receiving stimulation showed no such shift, implying that the spike-triggered stimulation produced plasticity, strengthening the connection between the recording site and either the stimulation site or downstream effectors.

5. MAPPING FUNCTIONAL CIRCUITRY

'Collision test' techniques using microstimulation to identify neurons sending axons to a specific area [73], or neurons receiving direct input from an area [74] allow coupling of *in vivo* electrophysiology with information about the specific inputs and outputs of the neuron being recorded. Sommer & Wurtz used these methods to explore the signals passed between three interconnected oculomotor brain areas (reviewed in [75]). In one such study, electrodes were placed in the FEF, the SC and the mediodorsal (MD) nucleus of the thalamus. The FEF sends projections to the SC, which in turns sends projections back to the FEF via MD. To identify neurons in MD receiving direct, monosynaptic input from the SC, microstimulation was applied to the SC while recording from a neuron in MD. Such monosynaptic, orthodromically evoked spikes should be of short latency, but slightly jittered relative to the microstimulation timing as a result of the variable delay introduced by synaptic transmission. By contrast, FEF microstimulation should antidromically activate MD neurons, which send projections to FEF, evoking a short and fixed latency action potential. Using this

technique they were able to identify neurons in the MD thalamus that transmit information from the SC to the FEF, as well as the SC and FEF neurons that send and receive this information, respectively. Recording from these identified neurons during various oculomotor tasks, they found that the SC sends short latency, spatially specific presaccadic activity to the FEF by way of MD, suggesting that the major signal conveyed by the pathway is corollary discharge information about the vector of impending saccades.

The combination of microstimulation and fMRI also allows investigation of the connectivity of distributed groups of neurons that may be functionally specialized. For example, this approach was used to investigate the face-selective patches within ventral visual cortex. Previous histology showed that certain portions of area TEO project to a handful of discrete areas within area TE [76], but could not provide any information regarding the visual selectivity of cells in these innervated regions compared with the surrounding cortex. Multiple fMRI studies have identified face-selective regions within the macaque temporal lobe, but have left open the question of whether these areas are interconnected [22]. By combining microstimulation and fMRI, Moeller *et al.* were able to examine connectivity and selectivity simultaneously [77]. Microstimulation of fMRI-localized face-selective patches of macaque visual cortex produced activation of the other face-selective areas, whereas microstimulation of the nearby temporal visual cortex produced an activation pattern that spared these face areas. This result suggests that face-selective regions of the temporal cortex are interconnected, perhaps allowing for specialized processing of these socially salient stimuli.

6. RECENT WORK AND FUTURE DIRECTIONS
(a) *Effects of varying pulse train timing*
A debate continues regarding the importance of precise timing of spikes over short timescales (e.g. [78] versus [79]), and the relative timing of spikes between multiple neurons (e.g. [80] versus [81]). Kimmel & Moore [82] examined the effect of varying patterns of current-pulse timing on the probability and metrics of saccades evoked via microstimulation of the FEF. Critically, the average pulse rate was held constant over a 35 ms window, a shorter window than the timescale over which the rate of neural firing is usually assumed to be averaged [78]. Although each 35 ms window of microstimulation always contained eight biphasic current pulses, the arrangement of these pulses within that window varied. Interpulse-intervals (IPIs) could be decreasing (accelerating), increasing (decelerating), random or uniform (fixed) (figure 4*a*). Kimmel & Moore found that the different pulse train patterns displayed disparate efficacy in evoking saccades. At a given current, eight pulses in 35 ms arranged in the accelerating pattern evoked saccades more frequently than if the eight pulses were delivered with a fixed IPI, which in turn were more effective than the eight pulses arranged in the decelerating pattern (figure 4*b*). This result shows that the oculomotor system is highly sensitive to the relative timing of these eight pulses within a narrow time window: simply rearranging the order of these seven
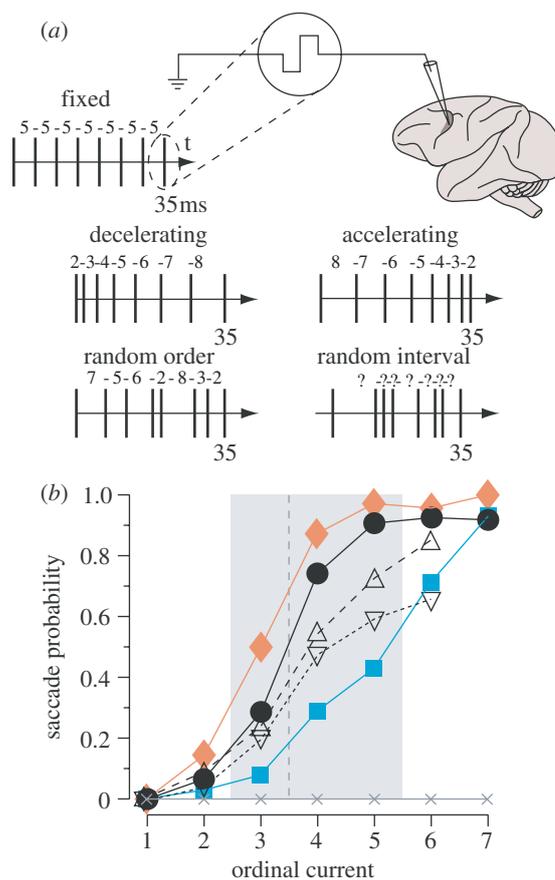

Figure 4. Varying the temporal pattern of microstimulation. (*a*) FEF was stimulated with pulse trains of 35 ms duration and a total of eight bimodal pulses; individual IPIs, in milliseconds, are displayed above each train (question marks denote random IPIs). IPIs were uniform (fixed, black circles), increasing (decelerating, blue squares), decreasing (accelerating, red diamonds), random order (RO, triangles), or random intervals (RIs, inverted triangles). (*b*) Average probability of evoking a saccade as a function of ordinal current (relative to site threshold, depicted by grey dotted line) at 14 FEF sites. All pairwise comparisons between patterns over the middle range of currents (shaded in grey) yielded significant differences except the two random patterns. Grey crosses, no stimulation. Adapted from [82].

IPIs is sufficient to alter the impact of microstimulation on behaviour.

One can also use microstimulation through multiple electrodes to examine the effects of relative timing of activation of two populations of neurons (this technique has yet to be attempted in primates). One recent study in cats showed that synchronous versus asynchronous stimulation of two populations of SC neurons resulted in different saccade vectors [83]. Synchronous stimulation resulted in averaging of the vectors represented by the two sites, while a temporal offset of as little as 5–10 ms between trains produced saccades closer to the sum of the two individual vectors. Another study directly probed the sensitivity of the nervous system to small timing differences between the activation of two neural populations in auditory cortex [84]. Rats were trained to respond based on whether the two sites were stimulated simultaneously or sequentially, and were able to reliably discriminate simultaneous versus sequential stimulation down to inter-stimulus intervals (ISIs) of

only 3–5 ms. Further work [85] indicates that the ISI discrimination threshold varies in different brain areas, presumably bearing some relation to the relevance of such timing differences for information processing in that region during normal behaviour.

(b) Stimulating single neurons

Researchers who wish to investigate the impact of single neurons on sensation and behaviour can use 'juxtacellular stimulation,' which involves precise positioning of the electrode near the cell membrane, in combination with currents so low that only a single neuron will be activated [86]. This approach can be used to study the effect of exciting neurons with different response properties, of different classes or located in different layers of cortex. Houweling & Brecht [87] used juxtacellular stimulation in rat somatosensory cortex to demonstrate that animals could detect activation of a single somatosensory neuron. A similar approach has even been used to examine the role of single tactile afferents in humans [88], where activation of a single tactile fibre produces a percept consistent with that fibre's tactile receptive field and preferred stimulus (e.g. vibration versus sustained indentation). These studies demonstrate that direct activation of even a single neuron can produce significant sensory and motor consequences.

(c) Future directions

For decades, microstimulation has been the predominant method of activating neurons *in vivo*, and thus one of the main means of drawing causal connections between neural activity and function. New techniques—for example optogenetics [89]—will soon allow researchers to target more specific subsets of neurons, enabling a finer scale dissection of neural circuitry than microstimulation can provide, and one of these methods may eventually supplant microstimulation as the technique of choice for causally manipulating neural activity. Despite activating neurons in such a comparatively non-specific manner, microstimulation has been remarkably useful in establishing the role of particular brain areas in perception, movement and cognition. Although one hopes that the coming years will establish more experimentally precise tools for probing neural circuitry and function, studies employing microstimulation have already contributed much to our understanding of the neural basis of behaviour, and their results will guide future work employing more sophisticated methods.

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