Direct and Indirect Activation of Cortical Neurons by Electrical Microstimulation

E. J. Tehovnik,¹ A. S. Tolias,² F. Sultan,³ W. M. Slocum,¹ and N. K. Logothetis²
¹Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts; ²Max Planck Institute for Biological Cybernetics, Tuebingen; and ³Department of Cognitive Neurology, Hertie-Institute for Clinical Brain Research, University of Tuebingen, Tuebingen, Germany

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Tehovnik, E. J., A. S. Tolias, F. Sultan, W. M. Slocum, and N. K. Logothetis. Direct and indirect activation of cortical neurons by electrical microstimulation. J Neurophysiol 96: 512–521, 2006; doi:10.1152/jn.00126.2006. Electrical microstimulation has been used to elucidate cortical function. This review discusses neuronal excitability and effective current spread estimated by using three different methods: 1) single-cell recording, 2) behavioral methods, and 3) functional magnetic resonance imaging (fMRI). The excitability properties of the stimulated elements in neocortex obtained using these methods were found to be comparable. These properties suggested that microstimulation activates the most excitable elements in cortex, that is, by and large the fibers of the pyramidal cells. Effective current spread within neocortex was found to be greater when measured with fMRI compared with measures based on single-cell recording or behavioral methods. The spread of activity based on behavioral methods is in close agreement with the spread based on the direct activation of neurons (as opposed to those activated synthetically). We argue that the greater activation with imaging is attributed to transynaptic spread, which includes subthreshold activation of sites connected to the site of stimulation. The definition of effective current spread therefore depends on the neural event being measured.

INTRODUCTION

Electrical stimulation of neural tissue has been in use for over 100 yr (at least since 1870, Fritch and Hitzig) and, although some have argued it is an imprecise technique for understanding the detailed mechanisms underlying different neural computations, microstimulation has actually contributed to important successes both in clinical applications and in uncovering the secrets of how the brain mediates various psychological processes. For example, electrical stimulation has made it possible to restore hearing to deaf patients by delivering microampere pulses by implanted electrodes to different regions of the cochlea (Bierer and Middlebrooks 2002, 2004; Fu 2005; Middlebrooks and Bierer 2002; Snyder et al. 2004). Stimulation of the basal ganglia has been remarkably effective in restoring motor function to Parkinsonian patients (Dostrovsky and Lozano 2002; Dostrovsky et al. 2000; Limousin et al. 1995; MacKinnon et al. 2005). In the same vein, microstimulation of the visual pathway is currently regarded as a very promising method for making the blind see again (Bartlett et al. 2005; Bradley et al. 2005; DeYoe et al. 2005; Merabet et al. 2005; Pezaris and Reid 2004; Schmidt et al. 1996; Tehovnik et al. 2005a; Zrenner 2002).

Equally important is the contribution of microstimulation in suggesting causal links between brain structures and behavior. Stimulation has been used to study pathways in the brain that subserve reward (Gallistel et al. 1981), as well as pathways involved in locomotion and startle responses (Yeomans and Frankland 1996; Yeomans and Tehovnik 1988). Its use has disclosed topographic maps in primary visual cortex (area V1), the supplementary eye fields, frontal eye fields, and the superior colliculus for the generation of ocular responses (Robinson 1972; Robinson and Fuchs 1969; Schäfer 1988; Tehovnik and Lee 1993). Additionally it has led to the elucidation of topographic maps in the motor and supplementary motor areas for the execution of skeletonmotor responses (Fritch and Hitzig 1870; Graziano et al. 2002; Penfield and Boldrey 1937; Strick and Preston 1978; Woolsey et al. 1952, 1979). Finally, electrical stimulation has been used to study cortical function as it pertains to the sense of vision, hearing, and touch (Britten and van Wezel 1998; DeAngelis et al. 1998; Penfield and Boldrey 1937; Penfield and Perot 1963; Romo et al. 1998; Salzman et al. 1990).

Many investigators are currently using electrical microstimulation routinely in behaving monkeys to make inferences on how neocortex mediates a range of behaviors, from target selection to avoidance responses (e.g., Cooke et al. 2003; Cutrell and Marrocco 2002; Moore and Armstrong 2003; Moore and Fallah 2001; Oppel et al. 2005; Schiller and Tehovnik 2001; Tehovnik et al. 2005a). In all such studies it is important to characterize the neural circuits that are activated during microstimulation both locally about the electrode tip and in projection sites. In this regard, two issues are of paramount importance: the need of accurate estimates of effective current spread and its effects on the excitable elements of the tissue. Our review therefore discusses both issues based on single-cell recording, behavioral methods, and neuroimaging.

EFFECTIVE CURRENT SPREAD USING DIRECT ACTIVATION OF CORTICAL NEURONS

Spread and excitability properties

It is commonly accepted that the sites of direct activation of a neuron with electrical microstimulation are at the initial segment and nodes of Ranvier (Gustaffson and Jankowska 1976; Nowak and Bullier 1998a,b; Porter 1963; Rattay 1999; Swadlow 1992). These zones contain the highest concentrations of sodium channels, thereby making them the most excitable segments of a neuron (Catterall 1981; Nowak and Bullier 1998a,b; Waxman and Quick 1978). The amount of
current injected through a microelectrode to directly activate a neuron (i.e., cell body or axon) is proportional to the square of the distance between the neuron and the electrode tip. This is expressed as \( I = K r^2 \), where \( I \) is the current level (in microamperes [\( \mu A \)]), \( r \) is distance (in millimeters [\( mm \)]), and \( K \) is the excitability constant (in \( \mu A/mm^2 \)). This relationship is derived from studies of cortical and corticospinal neurons of rats, cats, and primates (Asanuma et al. 1976; Marcus et al. 1979; Nowak and Bullier 1996; Shinoda et al. 1976, 1979; Stoney et al. 1968); dopaminergic fibers of the medial forebrain bundle in rats (Yeomans et al. 1988); cerebellar and reticulospinal fibers of rats, rabbits, and cats (Akaike et al. 1973; Armstrong et al. 1973b; Hentall et al. 1984a; Jankowska and Roberts 1972; Roberts and Smith 1973); cell bodies of cat spinal motor neurons (Gustaffson and Jankoska 1976); and axons of spinal interneurons of cats (Jankowska and Roberts 1972). In these studies, a single cathodal pulse was used to evoke an antidromic extracellular action potential as the stimulating electrode was advanced toward and beyond the element being stimulated.

The effective current spread from an electrode tip can be expressed as the square root of the current divided by the square root of the excitability constant, i.e., \( (I/K)^{1/2} \). This relationship is illustrated in Fig. 1A for a group of pyramidal tract neurons that were identified antidromically by pontine stimulation in cats (Stoney et al. 1968). To activate a neuron from the cortex, a 0.2-ms cathodal pulse was delivered through a microelectrode (Fig. 1, methods). The amount of current required for the evocation of an action potential 50% of the time defined the current threshold. As the electrode was advanced toward and past the neuron, the current threshold dropped and then increased accordingly (Fig. 1, methods, electrode S: a–c). The rate of change of the current threshold with electrode displacement was used to deduce the excitability constant. For the group of pyramidal tract neurons, the average excitability constant was 1,292 \( \mu A/mm^2 \). This constant reflects...
the excitability of a neural element 1 mm away from the electrode tip such that an element having a constant of 1,292 μA/mm² would require a 1,292-μA current to be activated 1 mm away 50% of the time. Increases in the excitability constant have been associated with decreases in the conduction velocity of an axonal element (Hentall et al. 1984a; Jankowska and Roberts 1972; Nowak and Bullier 1996; Roberts and Smith 1973). The conduction velocities of myelinated pyramidal tract neurons range from 3 to 80 m/s with the largest of these neurons exhibiting the highest velocities (Calvin and Sypert 1976; Deschenes et al. 1979; Finlay et al. 1976; Macpherson et al. 1982; Phillips 1956; Takahashi 1965); the conduction velocities of small unmyelinated cortical fibers are <1 m/s (Nowak and Bullier 1996; Swadlow 1985). The excitability constant derived with a 0.2-ms pulse can be as low as 300 μA/mm² for the largest myelinated cortical neurons and as high as 27,000 μA/mm² for the smallest unmyelinated cortical neurons (Nowak and Bullier 1996; Stoney et al. 1968). In other words, this constant is inversely related to the size of a neuron’s axon and to whether it is myelinated.

Strength–duration functions and estimates of excitability

To deduce the excitability of stimulated neurons, current can be traded off against pulse duration to elicit some response (Armstrong et al. 1973a; Asanuma et al. 1976; Bartlett et al. 2005; BeMent and Ranck 1969; Brindley and Lewin 1968; Dobelle and Mladjevsky 1974; Farber et al. 1997; Grumet et al. 2000; Hentall et al. 1984b; Jankowska and Roberts 1972; Li and Bak 1976; Matthews 1977; Nowak and Bullier 1998a; Ronner and Lee 1983; Rushton and Brindley 1978; Sekirnjak et al. 2006; Shizgal et al. 1991; Stoney et al. 1968; Swadlow 1992; Tehovnik and Lee 1993; Tehovnik and Sommer 1997; Tehovnik et al. 2003; Tolias et al. 2005; West and Wolstencroft 1983; Yeomans et al. 1988). This procedure is used to generate a strength–duration function. Normalized strength–duration functions for pyramidal tract neurons are illustrated [Fig. 1B, derived from Asanuma et al. (1976) and Stoney et al. (1968)]. As the pulse duration is increased, the amount of current needed to evoke an action potential 50% of the time diminishes to some asymptotic level; this level is called the rheobase current. The excitability or chronaxie of a stimulated element is expressed as the pulse duration at twice the rheobase current. The range of chronaxies for pyramidal tract neurons is illustrated in Fig. 1B in gray. These neurons exhibit chronaxies ranging between 0.1 and 0.4 ms.

The shorter the chronaxie the more excitable a directly stimulated neural element. Axons have shorter chronaxies than those of cell bodies (axons: 0.03–7 ms; cell bodies: 7–31 ms; Nowak and Bullier 1998a; Ranck 1975), and large, myelinated axons have shorter chronaxies than those of small, nonmyelinated axons (large: 0.03–0.7 ms; small: >1.0 ms; Li and Bak 1976; Ranck 1975; West and Wolstencroft 1983). A chronaxie is negatively correlated with the conduction velocity of axons (Nowak and Bullier 1998a; Swadlow 1992; West and Wolstencroft 1983) and positively correlated with their refractory period (Shizgal et al. 1991). The chronaxie is related to the time constant of the directly stimulated membrane of a neuron (Ranck 1975), which depends on a membrane’s resistance and capacitance (Bostock 1983; Bostock et al. 1983).

Effective current spread using behavioral methods

Spread properties

Several investigators have used behavioral methods to estimate how far current activates neural tissue mediating behaviors such as eating (Olds 1958), self-stimulation (Fouriez and Wise 1984; Milner and Laffarriere 1986; Wise 1972), and lateral head and body movements (Yeomans et al. 1986). These estimates are based on the activation of subcortical fibers. Two groups have studied the current-spread properties of electrical stimulation within neocortex using behavioral methods. Murasugi et al. (1993) studied such properties in extrastriate area MT (middle temporal cortex) and Tehovnik et al. (2004, 2005b) conducted current-spread studies in striate area V1.

Murasugi et al. (1993) stimulated area MT of monkeys with 1-s trains composed of 0.2-ms pulses delivered at 200 Hz to bias a monkey’s discrimination of the direction of dot motion. The motion stimuli were presented in the receptive field of the stimulated neurons, which were tuned to a particular direction of motion. Murasugi et al. found that for the range of currents tested (i.e., 10 to 80 μA), currents >20 μA biased a monkey’s discrimination abilities less well and began to obscure performance. Thus it can be presumed that ≤20 μA activates neural tissue confined to roughly one “directional” column in MT. The approximate width of such a column is about 0.2 mm (Albright et al. 1984). The average excitability constant of the activated elements in MT is therefore estimated to be 2,000 μA/mm² [K = 20 μA/(0.1 mm)²]. Finally, for pulse frequencies as high as 500 Hz (using 10-μA current pulses), a monkey’s performance on the discrimination task was never obscured; this suggests that such high frequencies delivered in 1-s trains exhibit effects confined to 0.2 mm of cortical tissue.

Tehovnik et al. (2004) found that microstimulation of V1 (with 100-ms trains using 0.2-ms pulses delivered at 200 Hz) systematically delayed the execution of visually guided saccades as long as the stimulation was delivered immediately before a monkey generates the saccade (i.e., at the end of the fixation period before the onset of the visual target) and as long as the visual target was punctate (<0.4° of visual angle) and located within the center of the receptive field of the stimulated neurons. No delay effect occurred to targets located outside of the receptive field of the stimulated neurons. It was later found that the size of the visual field affected by the stimulation, called a delay field, varied as a function of the site of stimulation within the operculum of V1 and it also varied as a function of current (Tehovnik et al. 2005b). A summary of the data from this study is illustrated in Fig. 2, A and B. As the stimulating electrode was situated further from the foveal representation of V1 the size of the delay field increased. For stimulations of cells having receptive field centers at 2, 3, and 4° from the fovea, the size of the delay field was 0.14, 0.24, and 0.35° of visual angle when using 100 μA and 0.09, 0.19, and 0.29° of visual angle when using 50 μA, respectively. The size of the visual field affected by stimulation can yield an estimate of how far the current spreads in V1 by using the visual magnification factor for V1 to convert size of visual field to area of tissue responsive to that field (see caption of Fig. 2C for detailed calculation). The amount of V1 tissue activated with...
A 50- and 100-μA current was estimated to be 0.572 and 0.736 mm, respectively, yielding a spread of 0.286 and 0.368 mm from the electrode tip. The average excitability constant of the directly activated elements in V1 is therefore estimated to be $675 \mu A/mm^2 \left[ K = \{50 \mu A/(0.286 \text{ mm})^2 + 100 \mu A/(0.368 \text{ mm})^2 \}/2 \right]$. Using this value, the distance of the effective spread of stimulation in V1 is plotted as a function of current ranging from 10 to 1,000 μA (Fig. 2C).

### FIG. 2

Using the stimulation-evoked delay of visually guided saccades in monkeys to make inferences about current spread and neuronal excitability in the primary visual cortex (area V1). A: latency difference between stimulation and nonstimulation trials for the generation of visually guided saccades to the target is plotted as a function of target eccentricity with respect to the receptive-field (RF) location of the stimulated neurons for 100 μA. A zero eccentricity along the x-axis indicates that the target and the RF center of the stimulated neurons were in register (see icon at the bottom right of the figures). Negative values along the x-axis indicate target positions situated between the fixation position and RF center of the stimulated neurons. Positive values indicate target positions eccentric to the RF center of the stimulated neurons. Noting the eccentricity value at 50% of the maximal delay for both negative and positive eccentricity values and computing an average, which was 0.49° of visual angle in this case, determined the size of a delay field. Parameters of stimulation were as follows: anode-first biphasic pulses were used and current, train duration, pulse duration, and frequency were fixed at 100 μA, 100 ms, 0.2 ms, and 200 Hz, respectively. These parameters (including the use of anodal pulses over cathodal pulses) were selected to optimize the delay effect (see Tehovnik et al. 2004). Anodal pulses are most effective at activating cell bodies and terminals, whereas cathodal pulses are most effective at activating axons even though in both cases it is the outward current at the initial segment or nodes of Ranvier that excite a neuron (Armstrong et al. 1973b; McIntyre and Grill 2000; Porter 1963; Ranck 1975; Rattay 1999).

The RF center of the stimulated units was at 4.3° from the fovea. Target used was brighter than background at 33% contrast (using the Michaelson contrast equation) and was 0.2° in diameter. Data from Tehovnik et al. (2005b). B: size of a delay field is plotted as a function of the eccentricity of the RF center of the V1 cells stimulated for 2 levels of current: 50 and 100 μA. Delay-field data points were fitted to linear functions. Stimulation sites were located from 0.9 to 2 mm below the cortical surface. Parameters of stimulation are indicated in A. Data from Tehovnik et al. (2005b). C: distance of effective current spread is plotted as a function of current. Function is derived from the data in B as follows: amount of visual field affected by 50 and 100 μA was used to infer the amount of tissue activated at V1 sites coding for 2, 3, and 4° of visual field eccentricity. Based on this, the amount of tissue activated in V1 was determined by noting the magnification factor for V1 sites coding for 2, 3, and 4° of visual field eccentricity. Amount of tissue activated per current level was then used to estimate the average $K$ (i.e., $675 \mu A/mm^2$) using the current–distance equation, $K = I/(R^2)$ (see Fig. 1A). Following is a detailed calculation for the derivation of a single $K$ value from the data in B: at an eccentricity of 3°, the size of the delay field was 0.24° using 100 μA. Magnification factor at this eccentricity is 0.32/mm, on average (Dow et al. 1981; Hubel and Wiesel 1974; Toottel et al. 1988). Therefore the estimated current spread at this V1 site is 0.375 mm [$0.24°/0.32\text{mm} \times 1/2$]. Current–distance equation yields a $K$ value of 711 μA/mm° [$100 \mu A/(0.375 \text{ mm})^2$]. D: current threshold normalized to the rheobase current $I_{th}$ is plotted as a function of pulse duration. Rheobase current is defined here operationally as the current used at the longest pulse duration (i.e., $0.7 \text{ ms}$). Each of 10 curves represents the current threshold for inducing a 20-ms increase in latency for saccades generated to a target located at the RF location of the stimulated neurons. Latency difference of 20 ms is >3 SDs of the mean difference observed when comparing nonstimulation and dummy stimulation trials (SD = 4.3, n = 35; Tehovnik et al. 2004). Each curve shows data from a single stimulation site. Sites were located from 0.5 to 1.5 mm below the cortical surface. See A for details about the electrical stimulation and visual target parameters. Pulse duration at which a curve intersects 2 units of threshold (designated by the dotted horizontal line) indicates the chronaxie of the stimulated elements at a site of study. Shaded region indicates the range of chronaxies of the stimulated elements. Data from Tehovnik et al. (2004). Methods (left): for all trials, a monkey was required to fixate (fix) a fixation spot for 600 ms. At the termination of the fixation spot, a visual target appeared (targ) and the monkey was required to generate a saccadic eye movement (sacc) to the target to obtain a juice reward (juice). On 50% of trials electrical stimulation (stim) was delivered at the end of the fixation period to determine whether the stimulation delayed the visually guided saccade. Methods (right): visual target was presented at various locations with respect to the fixation spot (fix) and RF center of the stimulated neurons.
Excitability properties

The excitability properties of stimulated neurons mediating a variety of behaviors including self-stimulation (Matthews 1977), conditioning (Bartlett et al. 2005), ocular responses (Tehovnik and Lee 1993; Tehovnik and Sommer 1997; Tehovnik et al. 2003, 2004), and phosphen induction (Brindley and Lewin 1968; Dobelle and Mladjeovisky 1974; Rushton and Brindley 1978) were determined by generating strength-duration functions. As discussed, strength-duration functions yield the chronaxies of the activated neurons eliciting the stimulation-evoked response. It is commonly assumed that chronaxies derived using behavioral measures reflect the current integration characteristics of the directly stimulated elements and not those of the follower cells or innervated muscles (for a review of arguments see Gallistal et al. 1981; also see Tehovnik 1987). Chronaxies have been deduced for V1 elements inducing the delay of visually guided saccades (Tehovnik et al. 2004). Recall that Tehovnik et al. (2005b) used this behavioral response to determine the current-spread properties of stimulation delivered to V1 cortex. The chronaxies of the elements mediating the delay of saccades ranged between 0.1 and 0.3 ms (Fig. 2D), which overlaps with the chronaxies described for pyramidal tract fibers (Fig. 1B). Therefore, the stimulated neurons that mediate the delay effect are probably composed of the largest and most excitable fibers that reside in V1. This is consistent with the relatively low excitability constant found for the V1 elements mediating this effect (K = 675 μA/mm²).

EFFECTIVE CURRENT SPREAD USING fMRI

Spread properties

Recently, Tolias et al. (2005) used fMRI (functional magnetic resonance imaging) that relies on the BOLD (blood oxygen level–dependent) signal to study the spread properties of electrical microstimulation delivered to V1 cortex in the anesthetized monkey. An anesthetized preparation was used to avoid motion artifact, which would have greatly compromised the accuracy of the current-spread estimates. All stimulations of V1 were conducted through microelectrodes positioned within the gray matter of V1; electrode-tip positions were validated using anatomical MRI. The electrically evoked BOLD response was measured using a 4.7-Tesla scanner. To evoke a BOLD response, a 4-s train of stimulation composed of 0.2-ms pulses delivered at 100 Hz was used. The train duration of 4 s concurs with the duration of visual stimulation used to evoke a fMRI response from V1 (Logothetis et al. 1999, 2001). Figure 3A shows that for current levels of 159 to 1,651 μA some 2 to 4.5 mm of cortical tissue was activated from the electrode tip. For currents >458 μA the spread began to saturate between 3 and 5 mm. The extensive spread of current as measured with fMRI is consistent with the spread observed using optical imaging of neocortex where lower currents were used (Seidemann et al. 2002; Slovin et al. 2003).

Excitability properties

Tolias et al. (2005) determined strength–duration functions for the activation of 40% of the maximal volume of gray matter within the operculum using fMRI in anesthetized macaque monkeys. Some five sites were studied using five monkeys.Currents from 200 to 1,600 μA and pulse durations from 0.05 to 0.6 ms were tested. The chronaxies of the stimulated elements yielding the stimulation-elicited fMRI response averaged 0.18 ms (Fig. 3B). This value suggests that the evoked fMRI response does not arise from the activation of relatively unexcitable tissues such as smooth muscle, which exhibit chronaxies between 6 and 13 ms (Sibley 1984). The value coincides with the chronaxies reported for pyramidal fibers (see Fig. 1B). Therefore the fMRI response generated by activation of macaque V1 is likely explained by the excitation of the most excitable cortical neurons.

DISCUSSION

This review describes the excitability of neurons and effective spread of electrical microstimulation in neocortex using three different methods: 1) single-cell recordings of antidromically identified cortical neurons that were activated directly by the stimulation; 2) behavioral methods used to infer the cortical spread of the current required to evoke a behavioral response such as biasing a monkey’s visual behavior by MT stimulation or delaying a monkey’s visually guided saccades by V1 stimulation; and 3) imaging methods that measured the extent of neural activation about a stimulating electrode positioned in cortical area V1. The three methods produced very similar strength–duration functions yielding chronaxie values that overlapped and fell between 0.1 and 0.4 ms (cf. Figs. 1B, 2D, and 3B). In other words, behavioral and imaging methods corroborate the electrophysiological evidence that electrical microstimulation activates the most excitable elements in neocortex, that is, primarily the pyramidal fibers (Asanuma et al. 1976; Stoney et al. 1968). The three methods, however, produced different current-spread estimates for cortical stimulation. Single-cell recording and behavioral methods yielded estimates that were comparable (Fig. 3A, cf. T, S, M). fMRI, on the other hand, yielded estimates roughly fourfold greater than those observed using the other methods (Fig. 3A, compare fMRI “histogram” with functions T, S, and M).

An obvious reason that could account for the observed difference between the fMRI data and the other measures of effective current spread is the appreciably larger currents and longer train durations used in the fMRI study (Tolias et al. 2005). In the unit recording and behavioral experiments the current was <150 μA and the train duration was ≤1 s, whereas in the fMRI experiments the current was greater, typically falling between 150 and 2,000 μA with train durations ≤4 s. To compare the two data sets, the current-spread data of the unit recording and behavioral experiments had to be extrapolated into the milliamper range using the current–distance equation, I = Klr² (Fig. 3A). It is possible that the greater functional spread of activation with fMRI does not hold at low currents and short train durations. However, Seidemann, Slovin, and colleagues delivered low currents (<100 μA) and short train durations (<250 ms) to neocortex and found activation far beyond the field of direct stimulation when measured with optical imaging (Seidemann et al. 2002; Slovin et al. 2003). Moreover, Slovin et al. (2003) reported that a single pulse of 15 μA can activate cortical tissue between 1.5 and 3 mm from the electrode tip. Therefore the greater spread of activation obtained with fMRI might instead be related to transynaptic activation.
Two observations support this notion. First, V1 is composed of columns that are interconnected by horizontal projections (Gilbert and Wiesel 1989). In monkeys, these projections extend laterally by 1 to 4 mm (Blasdel et al. 1985; Fisken et al. 1973; Fitzpatrick et al. 1985; McGuire et al. 1991). This distance concurs with the lateral spread of the fMRI response, which is 1.5 to 3.5 mm beyond the fringe of direct stimulation (Fig. 3A, compare fMRI “histogram” with function S). This maximal extent corresponds to the maximal extent of the horizontal projections in V1 and suggests that horizontal projections within cortex might put an upper limit on how much tissue is activated by focal electrical stimulation when using fMRI.

Second, stimulation of V1 is found to activate regions of extrastriate cortex that are connected monosynaptically with V1 as measured with fMRI. Such stimulation elicits the fMRI response in V2, V3, V3A, V4, and MT with noticeable gaps in activity between the site of stimulation in V1 and its extrastriate targets (Tolias et al. 2005). These findings agree with the known anatomical connections between V1 and the extrastriate cortex (Lund et al. 1975; Maunsell and van Essen 1983; Perkel et al. 1986; Rockland and Pandya 1979, 1981; Sincich and Horton 2003; van Essen et al. 1986; Yukie and Iwai 1985; Zeki 1978). This transynaptic activation may be dominated by subthreshold responses over neuronal spiking (Akgo¨ren 1996; Logothetis et al. 2001; Mathiesen et al. 1998).

The larger than expected spread as measured with fMRI might also indicate the presence of spiking activity beyond the site of direct stimulation, however. It is well established that even a single electrical pulse delivered to neural tissue activates fibers transynaptically within a cortical structure (Asanuma and Rose´n 1973; Asanuma et al. 1976; Butovas and Schwarz 2003; Jankowska et al. 1975; McIlwain 1982; Stoney et al. 1968). A 4-μA (at 0.2-ms pulse duration) current pulse delivered to motor cortex can activate tissue laterally and transynaptically out to 1.5 mm from the electrode tip (Asanuma and Rosén 1973). Delivering a train of four 30-μA pulses (at 400 Hz) to the superior colliculus can activate tissue ±2 or 3 mm laterally and transynaptically from the electrode tip (McIlwain 1982).

One way of testing whether the lateral connections from the site of stimulation are activated directly is to measure the
spread of cortical activity from the stimulating electrode at various times after stimulation onset. If all the elements mediating the stimulation-induced response are excited simultaneously, this would suggest that all fibers are being activated directly. On the other hand, if the tissue closest to the electrode is activated first followed later by the tissue furthest from the electrode, this would suggest that the delayed response arises from indirect activation. Such lateral spread of activity triggered by a punctate visual target was demonstrated in V1 using optical imaging and attributed to conduction laterally by unmyelinated, synaptically connected fibers within cortex or by conduction through feedback projections from extrastriate cortex (Grinvald et al. 1994; Slovin et al. 2002). The lateral spread of activity was estimated to range from 0.09 to 0.25 m/s from the center of direct activation and to travel between 1 and 4 mm through the operculum.

If the lateral spread of activity is so prevalent in cortical tissue even at the lowest currents, why then does microstimulation of neocortex evoke precise behavioral responses as evidenced, for example, by the fine correspondence between the endpoint of an electrically evoked saccade from V1 and the center of the receptive field of the directly activated neurons (Tehovnik et al. 2003)? It is possible that the behavioral effects of microstimulation may be caused by decoding the activity of a neuronal population significantly larger than the neurons activated directly as a result of the passive spread of current. Yet it is thought that stimulation disproportionately activates the largest and most excitable elements of cortex directly and that these elements tend to project subcortically rather than laterally (Calvin and Sypert 1976; Deschenes et al. 1979; Finlay et al. 1976; Macpherson et al. 1982; Nowak and Bullier 1996; Phillips 1956; Stoney et al. 1968; Swadlow 1985, 1988; Takahashi 1965). These stimulated neurons might more readily gain access to subcortical networks involved in the execution of precise behavioral responses such as cortically generated saccadic eye movements. The chronaxies of the directly stimulated elements that mediate a variety of cortically evoked behaviors including saccadic eye movements, conditioning responses, and phosphenes induction fall between 0.1 and 0.4 ms (Bartlett et al. 2005; Brindley and Lewin 1968; Dobelle and Mladiejsky 1974; Rushton and Brindley 1978; Tehovnik and Lee 1993; Tehovnik and Sommer 1997; Tehovnik et al. 2003).

As mentioned earlier, such short chronaxies obtained with different methods suggest that microstimulation is directly activating the largest elements in cortex.

A second possibility of why microstimulation of neocortex produces precise behavioral responses is that the activation of the lateral projections in cortex might fail to significantly contribute to spiking activity and thus to an evoked behavior resulting from conduction issues. The lateral connections within cortex are often unmyelinated and therefore relatively unexcitable (Nowak and Bullier 1996; Swadlow 1985). Unmyelinated fibers are prone to conduction failure (Raymond and Lettvin 1978; Swadlow et al. 1980), whereas large myelinated fibers can follow pulse frequencies well in excess of 100 and ≈800 Hz (Macpherson 1982; Puntal 1965; Stoney et al. 1968; Swadlow 1985; Takahashi 1965). One consequence of this is that the directly activated tissue may contribute disproportionately to an evoked behavioral response. This supposition is consistent with the finding that directly activated fibers in V1 using a punctate visual stimulus result in a discharge of spikes, whereas laterally connected fibers exhibit postsynaptic potentials without spiking (Bringuier et al. 1999).

A third and related possibility of why microstimulation of neocortex evokes precise responses is that the neurons activated directly make a more significant contribution to the evoked response because they are more synchronously activated compared with the neurons further away from the electrode tip activated transynaptically in cortex (Tolias et al. 2005).

In conclusion, the established observation that electrical microstimulation of cortical tissue activates the most excitable cortical neurons receives support from studies using different methodologies, including neuroimaging. The greater lateral spread of the hemodynamic, fMRI signal, in the worst case, could denote the extent of subthreshold activation of nearby neurons, although spiking cannot be ruled out. At present one can only note that the definition of effective current spread necessarily depends on the measured quantity. We suggest that field potentials and neuroimaging are likely to give larger current-spread estimates because they reflect both dendritic spikes and perisynaptic events, such as population excitatory and inhibitory postsynaptic potentials, as well as afterpotentials. For the behaviors described in this review, it seems that the directly activated elements contribute disproportionately to the evoked behavioral response given the likeness of the effective current-spread estimates for the electrophysiological and behavioral experiments. We are hopeful that this review compels investigators to do more excitability and current-spread studies using a variety of techniques—i.e., fMRI, optical and two-photon imaging, multiple unit recording with arrays, and so on—to examine various behavioral systems so that we can make direct links between behavior and the biophysics of the brain.

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