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Functional reorganization of primary visual cortex induced by electrical stimulation in the cat

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Abstract

Compared to the high degree of plasticity observed in a juvenile, mature sensory cortices have long been held to be immutable but, recently, researchers have suggested some plasticity persists in the mature cortex. Cortical reorganization has particular saliency to the development of a cortically based, sensory neuroprosthesis, which will chronically evoke activity through electrical stimulation. We have examined the nature and extent of the reorganization induced by electrical stimulation. We found the receptive field size and synaptic efficacy can be increased, particularly for neurons near the stimulation site. As the changes are minimal, these results are not expected to impact neuroprosthetic applications.
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1. Introduction

Although it has been long held that the receptive field characteristics of neurons in mature sensory cortex are static, recent work indicates that these neurons' responses can change dynamically depending on the context of the stimulus (for a review see Gilbert, 1998). Changes in receptive field characteristics of neurons in primary visual cortex can result from stimuli outside their classic receptive fields (Freeman, Ohzawa, & Walker, 2001; Rossi & Paradiso, 1999) or from selective stimulation within their classical receptive fields (DeAngelis, Anzai, Ohzawa, & Freeman, 1995; Dragoi, Rivadulla, & Sur, 2001; Pettet & Gilbert, 1992). Additionally, electrical stimulation of sensory cortex has been shown to lead to receptive field changes (Maldonado & Gerstein, 1996a; Maldonado & Gerstein, 1996b; Recanzone, Merzenich, & Dinse, 1992; Spengler & Dinse, 1994) and particularly so when the electrical stimulation is tied to an external stimulus (Schuett, Bonhoeffer, & Hubener, 2001). More recently, changes in the orientation preference of neurons in primary visual cortex were seen as a result of electrical stimulation (Godde, Leonhardt, Cords, & Dinse, 2002). The emerging view is that mature sensory cortex maintains some degree of plasticity.

The electrically induced reorganization of sensory cortex has particular saliency to the implementation of a cortically based sensory neuroprosthesis, where relatively small numbers of neurons will be electrically stimulated in order to restore partially a lost sensory modality. For example, in a cortically-based vision neuroprosthesis, electrical stimulation of a single intracortical electrode produces the percept of phosphene, a small spot of light (Bak et al., 1990; Brindley & Lewin, 1968a; Dobelle & Mladejovsky, 1974). Due to the retinotopic organization of primary visual cortex (and assuming that phosphenotopy follows the retinotopic organization),

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one theoretically should be able to produce an ordered arrangement of phosphenes by stimulating visual cortex via an ordered arrangement of spatially distributed electrodes (Schmidt et al., 1996). However, if electrical stimulation induces considerable changes in the receptive field characteristics of the stimulated neurons and, most likely, the evoked phosphene's characteristics, the utility of the neuroprosthesis may be compromised or, at minimum, the design of such a device will have to accommodate large-scale reorganization.

We have investigated the potential for cortical reorganization by electrically stimulating neurons in cat primary visual cortex and monitoring the receptive field properties of the stimulated neurons and other, nearby neurons before and after electrical stimulation. Further, we compared the synaptic connectivity of these neurons before and after electrical stimulation via cross-correlation analysis of spontaneous activity. Although statistically significant changes in the receptive field size and synaptic efficacy were observed, these changes were minimal and are not anticipated to influence greatly the development and use of a cortically based vision neuroprosthesis. Most likely, the user of a clinical visual neuroprosthesis readily will accommodate to these fairly small changes.

2. Methods

The results described in this study were obtained in six anesthetized and paralyzed cats using techniques fully described elsewhere (Nordhausen, Maynard, & Normann, 1996; Warren, Fernandez, & Normann, 2001) and only briefly described here. Experiments were performed under animal care and experimental guidelines that conformed to those set by the National Institute of Health. Anesthesia was induced with either Telazol or ketamine. The animals were cannulated, intubated, and their heads immobilized. They were artificially ventilated and anesthesia was maintained with halothane (approximately 0.8–1.0% during recording). Visual cortex was exposed by a 1- to 2-cm diameter craniotomy and the dura reflected. We implanted an array of 100-penetrating electrodes (Jones, Campbell, & Normann, 1992) (Cyberkinetics Inc., Foxborough, MA) into striate cortex, at the junction of the lateral and posterior lateral gyri, with the majority of the tips of the electrodes implanted to the approximate depth of layer IV. In a single animal we verified the majority of the tips of the electrodes were localized to layer IV by histological examination (Warren et al., 2001) but, given the curvature of this layer in cat striate cortex, we cannot be certain that all electrodes in all animals were in layer IV. The electrodes were configured in a 10×10 grid with 400 μm spacing between electrodes. The array was allowed to move with the breathing-associated motion of the cortex, which, in our experience, enhances the ability to track units for long periods. The recording and stimulation reference was provided by a separate platinum-iridium wire inserted within 2 cm of the array and to the depth of the white matter. The pupils were dilated, the nictitating membranes were retracted, and the eyelids were sutured open. Gas permeable contact lenses were placed in each eye to protect the corneas. After a stable anesthetic plane was established, paralysis was initiated with pancuronium bromide (0.1 mg/kg/h, i.v.). The retinas were back refracted onto a tangent screen and the locations of retinal landmarks were recorded on the screen to locate area centralis (Bishop, Kozak, & Vakkur, 1962; Nikara, Bishop, & Pettigrew, 1968). Neural events, as well as external stimulus marker codes, were recorded with a 100-channel data acquisition system (Guillory & Normann, 1999) (Cyberkinetics Inc, Foxborough, MA).

2.1. Electrical stimulation

Only a small number of electrodes (2–5) were electrically stimulated in each animal to allow distant, unstimulated electrodes to act as controls. As an additional control, we performed a sham electrical stimulation protocol in one animal. Here we performed the same electrical stimulation procedures without connecting the current sources to the array. After this sham protocol, we performed an actual electrical stimulation protocol in the same animal. Stimulated electrodes were selected from the group of electrodes that appeared to have a robust response to the random checkerboard visual stimulus (described below). Additionally, we selected electrodes for stimulation that distributed the stimulated electrodes over the array while leaving some regions unstimulated. In five of the animals, the stimulus consisted of a train of biphasic current pulses delivered once a second for 4–9 h. Each biphasic pulse consisted of a 200 us cathodic phase, a 100 us interpulse interval, and a 200 us anodic phase. Each stimulus train consisted of 15 pulses delivered at 250 Hz. Both low (10–60 μA) and high (250 μA) current amplitudes were tested. This protocol is similar both to a protocol found effective in inducing plasticity in the cat visual cortex (Godde et al., 2002) as well as a test protocol used in a blind human volunteer (Schmidt et al., 1996). The current magnitude exceeds that necessary to induce plasticity in cat visual cortex, 6 μA, (Godde et al., 2002) and encompasses the range necessary to induce behavioral actions in both cat auditory cortex, 57–77 μA, (Rousche & Normann, 1999) and rat auditory cortex, 16.7–69.2 μA (Rousche, Otto, Reilly, & Kipke, 2003). The estimated region of stimulation is a sphere, centered at the electrode tip, and having a diameter of between 88 and 440 μm for 10–250 μA,

respectively (Stoney, Thompson, & Asanuma, 1968). In one animal, where we were attempting to induce kindling (Goddard, McIntyre, & Leech, 1969), the stimulus consisted of a train of biphasic current pulses delivered once an hour for 29 h. Each stimulus train consisted of 240 pulses delivered at 60 Hz. The individual pulses have the same temporal characteristics as described above. A current amplitude of 150 μA was used here. During electrical stimulation, the animal was held in the dark and, due to technical limitations, no data recordings were made.

2.2. Visual stimulation

We mapped the approximate location and orientation preference of the multi-unit neural response for each electrode using bars projected onto a tangent screen with a hand held projector. A computer monitor was placed at the approximate visual space representation of the majority of the receptive fields. In the first animal, we used a 15-in. monitor (Viewsonic Model 15GS), placed 90 cm from the eye. In the five later animals, we used a 17-in. monitor (Hitachi Model 620), placed 80 cm from the eye. Both monitors had a 640×480-pixel resolution and 100-Hz refresh rate. A random checkerboard pattern was presented on the monitor that consisted of a number of equal sized squares, each of which subtended $1.1° \times 1.1°$ ($1.0° \times 1.0°$ on the 15-in. monitor). In addition, the entire random checkerboard was shifted both vertically and horizontally by randomly, and independently, selecting the horizontal and vertical origin of the checkerboard as integer multiples of 0.14° (0.26° on the 15-in. monitor). In the first two animals, each checkerboard square was set randomly to either black or white with a 25% probability of being white. In the remaining animals, each checkerboard square was set randomly to one of three states, black, white, or gray, with probabilities of 15%, 15%, and 70%, respectively. A new checkerboard pattern with a new horizontal and vertical offset was displayed at a rate of 25 Hz. For each measurement of the receptive field properties (trial), a series of checkerboard stimuli was presented monocularly, with the other eye covered, and neural data recorded for 30 min. Each trial was followed approximately 30 min of both other visual stimulus tests, not reported here, and extended periods of recording where the visual stimulus was unchanging (the entire computer screen was set to a single intensity gray). Typically, five trials for each eye were performed before electrical stimulation, lasting a total 5 h if only the contralateral eye was examined and 10 h if both the contralateral and ipsilateral eyes were examined. After electrical stimulation, another two to five trials were made for each of the eyes tested before electrical stimulation as well as additional periods of recording where the visual stimulus was unchanging.

2.3. Data analysis

The single units on each electrode were separated using a mixture-of-t-distributions classification technique (Shoham, Fellows, & Normann, 2003). If a unit fired at least 100 times in at least half of the visual checkerboard stimulation trials, then the unit was considered reliable (or observed) and an examination of its receptive field properties warranted.

The reverse correlation method was used to estimate the receptive field properties from the response to the random checkerboard pattern (Eckhorn, Krause, & Nelson, 1993; Jones & Palmer, 1987). This was done by cross-correlating spike times with the visual stimulus over a range of latencies between the stimulus and the spike. The raw correlation data was normalized into t-statistics by subtracting out the average screen intensity and dividing out the standard deviation of the screen intensity on a pixel-by-pixel basis. The result of this calculation is a three dimensional representation of the receptive field map with one dimension of time and two dimensions of visual space. If the largest magnitude of the t-scores across all three dimensions exceeded 4.3 (approximately 1 in 10^5 chance of occurring if spikes are not related to the visual stimulus), then the receptive field measurement was considered reliable and the data examined further. The latency to the largest magnitude (peak latency) was found and subsequent analysis only used the spatial data at this latency. This two dimensional data was smoothed with a two-dimensional Gaussian filter having a 12 pixel standard deviation. From the smoothed data, the peak value was extracted. The border of the receptive field was obtained as the boundary of the contiguous region surrounding the peak value and having magnitude of 30% or greater than the peak. The receptive field size was defined as the size of the region within the border and the location was defined as the center of mass within the border.

Interspersed with periods of visual stimulation were extended periods where the entire computer screen was held at a gray level. During these times, we recorded spontaneous neural activity. Using these data, we extracted the cross-correlation using NeX (NeuroExplorer, Littleton, MA) in 3 ms wide bins over the range of ±250 ms. These data were calculated only with units having at least 100 isolated spikes (described below). To reduce the impact of global synchrony (Eggermont & Smith, 1995), we subtracted out the shift-predicted cross-correlation, where one of the neural spike trains was shifted by 1 s. Additionally, we only used isolated spikes to calculate the correlation where isolated spikes were defined as the spikes that followed the preceding spike by at least 10 ms and preceded the following spike by at least 10 ms (Eggermont & Smith, 1996). Further, the mean of the cross-correlation across all latencies was subtracted from the cross-correlation, making the

statistics more correctly labeled the cross-covariance. From these data, we calculated the correlation magnitude as the average correlation in a 1.5–10.5 ms window after (and separately before) simultaneous events.

Due to the longitudinal nature of these experiments, where we planned to examine changes in the neural response of single units tracked for up to 3 days and intended to identify single units based only upon the kinetics of the waveform of the extracellular action potential, we used a high degree of conservatism in our single unit classification and data analysis. If a single unit did not robustly respond to visual stimulation, did not exhibit statistically reliable waveform kinetics, or did not reliably generate a receptive field map in the approximate same location with similar temporal characteristics, we discarded the unit from the analysis. Although this greatly reduced the number of units, we believe the conservative approach was warranted due to the potential confound of ascribing a result to electrical stimulation that is actually due to other sources such as electrode movement. Was an electrode to move, it is possible that a new unit could be sensed that had similar waveform kinetics but different receptive field properties from the originally detected unit. However, as the electrode array is a rigid structure and movement at one electrode will likely result in movement of multiple electrodes, it is highly improbable for the array to have moved yet still measure similar waveform kinetics at multiple electrodes. Hence, it is highly unlikely that any observed receptive field property changes are due to electrode movement.

3. Results

3.1. General observations

The data described in this report come from experiments performed in six cats. In four of these experiments, we observed brisk responses from the recorded neurons when visually stimulating either eye. In these experiments, we collected data while visually stimulating the eye contralateral to the implant site (contralateral eye) and the eye ipsilateral to the implant site (ipsilateral eye), one eye at a time. In the remaining two experiments, we noted that only a small number of recorded neurons exhibited vigorous responses when visually stimulating the ipsilateral eye in the initial part of the experiment. Consequently, in these experiments we collected data only for the contralateral eye. Across the possible 562 electrodes sites (reduced from the theoretic possibility of 600 electrodes due to broken wires, electrodes, and amplifier channels), we extracted 1025 units, or around an average of two units per electrode. Of these units, 714 were observed both before and after electrical stimulation, 216 were observed only before electrical stimulation, and 95 were observed only after electrical stimulation. We were able to generate reliable receptive field maps for the majority the random checkerboard stimulus trials both before and after electrical stimulation with 196 of the 714 units observed throughout an experiment. The signal-to-noise ratio of these 196 units ranged from 1.1 to 8.9 with a median of 1.9. A summary of the number of observed units and the number of units with receptive fields on a per animal basis is provided in Table 1. In the longest experiment performed, we reliably generated receptive field maps for 45 units across a period of around 56 h. From the 22 electrode sites that were electrically stimulated, we extracted 38 units (which were included in the total of 1025 units given above). Nineteen of these units were observed both before and after electrical stimulation and 19 were observed only before stimulation. Of the 19 units observed both before and after electrical stimulation, we were able to generate reliable receptive field maps for the majority of the random checkerboard stimulus trials both before and after electrical stimulation with 5 units (which were included in the total of 196 units given above). Six of these 19 units reliably generated a receptive field before stimulation but not after stimulation. An additionally 5 of the 19 units had a sufficient level of spontaneous activity to allow derivation of a meaningful isolated-spike, cross-correlation both before and after electrical stimulation. The results presented in the following sections come from 201 out of the 1025 units; the 196 units that reliably generated a

Table 1
Number of units observed and with receptive field

Animal	Current level (μA)	Duration (h)	Number of units observed only before stimulation	Number of units observed only after stimulation	Number of units observed before and after stimulation	Number of units with contralateral RF	Number of units with ipsilateral RF	Number of units with binocular RF
1	60	4	22	40	105	18	9	5
2	150	29	39	9	99	22	18	5
3	250	9	49	27	89	19	0	0
4	250	9	50	11	52	6	0	0
5	25	6	40	4	123	15	9	7
6	10	5	16	4	246	42	15	6
All			216	95	714	122	51	23

receptive field map throughout an experiment and the 5 units where we could generate a meaningful cross-correlation throughout an experiment.

3.2. Single-unit receptive field properties

To investigate whether electrical stimulation induced changes in the functional organization of the neural circuit, we examined the single-unit properties of receptive field size and the magnitude of the response to visual stimulation. By comparing the nature and extent of the properties' change with electrical stimulation to the average observed prior to stimulation (in the context of the variability observed prior to stimulation), the significance of the relationship between electrical stimulation and changes was developed. If a unit had spatially distinct ON and OFF regions, the analysis was performed independently for each region. If a unit was binocular (and was associated with an animal wherein both contralateral and ipsilateral data were available), the analysis was performed independently for the contralateral and ipsilateral data.

An example of a single unit's receptive field maps from both before and after electrical stimulation, the extracted property of size, and the average firing rate, is shown in Fig. 1. This particular unit was recorded on an electrode that was electrically stimulated once a second for 5 h with a train of 15 biphasic pulses delivered at 250 Hz and having a current magnitude of 10 μA. Fig. 1A–F shows the receptive field maps from a series of trials, one trial per panel, with each map presented as a mesh plot of the smoothed version of the t-score data coming from the reverse correlation method. Only the data from the time latency giving the largest t-score magnitude are shown. All mesh plots have the same scaling in all three axes with the x- and y-axes portraying location in visual space, in degrees, and the z-axis portraying the unitless t-score. The upper row of mesh plots is from trials taken prior to electrical stimulation and the lower row is from trials taken after electrical stimulation. On each mesh plot, the border of the receptive field, defined as the contour at 30% of the peak value, is shown as a darker line. The receptive field size is defined to be the size of the region within this border. A comparison of the receptive field borders across trials is shown in Fig. 1G, with data from trials before electrical stimulation shown as light gray lines, the data from the first trial after electrical stimulation shown as a black line, and the data from the remaining trials after electrical stimulation shown as darker gray lines. A comparison of the unit's average firing rate across trials is shown in Fig. 1H, with data from before electrical

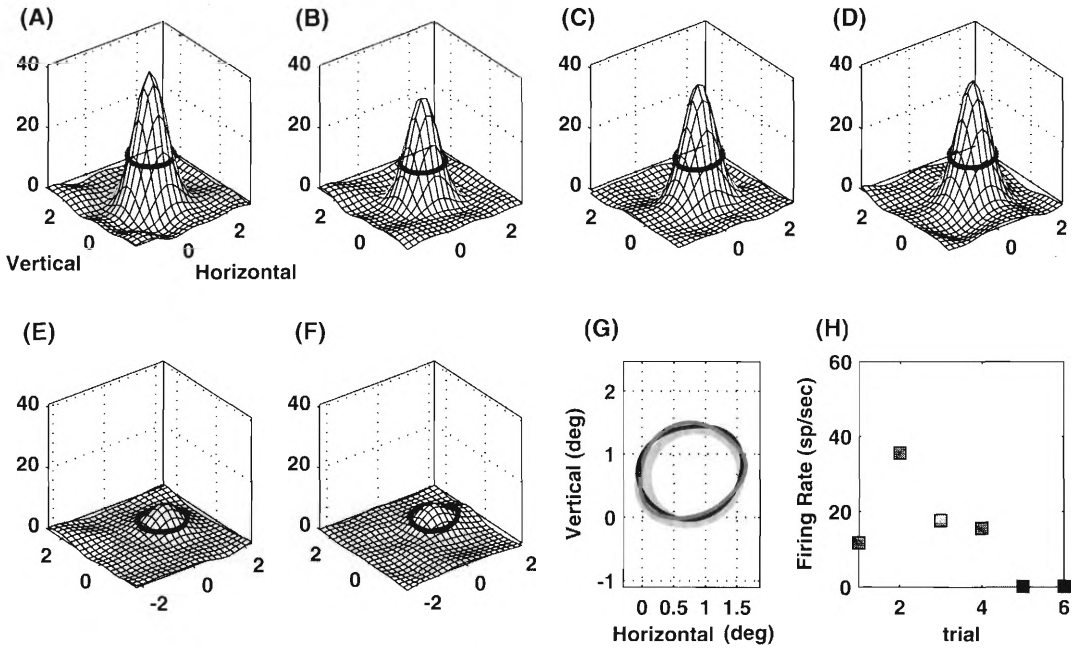

Fig. 1. Representative example of receptive field maps before and after electrical stimulation of a unit recorded on an electrically stimulated electrode. Panels A–D show the receptive field map, as a three-dimensional plot of the t-score derived from the reverse correlation method. The four measurements are relatively similar in the peak magnitude and the border of the receptive field, the later shown as the dark line in each panel. Panels E and F show the same data after electrical stimulation. A strong reduction in the peak value is clearly indicated. Panel G shows the border of the receptive field from both before stimulation (light gray lines) and after stimulation (black line for first trial after stimulation and dark gray otherwise). The borders are very similar and there is no statistical evidence to conclude the areas within these borders (receptive field size) differ between before and after electrical stimulation. Panel H shows the average firing rate before stimulation (light gray filled symbols) and after stimulation (black filled symbols).

stimulation shown as light gray filled symbols and the data from after electrical stimulation shown as symbols filled with black.

A number of features stand out in this figure. First, the measurement of the receptive field is relatively consistent prior to electrical stimulation as can be seen by comparing the mesh plots of the upper row. The magnitude of the peak t-score is similar for all four trials before electrical stimulation. The border of the receptive field also does not greatly change prior to electrical stimulation. This is best seen by comparing the almost indistinguishable four line gray lines in Fig. 1G. As the border does not appreciably change, the change in the receptive field size also is slight, ranging between 1.64 and 1.94 degree squared. The similarity of the receptive field properties in trials prior to electrical stimulation generalized to all units and animals. More specifically, there is no statistical evidence to conclude that any trial taken prior to electrical stimulation significantly differed from the other trials before electrical stimulation (ANOVA with repeated measures, $\alpha = 0.05$) when examining any of the receptive field size, peak t-score, or the average firing rate. Further, in the one animal where we performed a sham electrical stimulation protocol, there is no statistical evidence to conclude that the receptive field size or peak t-score from the trial after the sham electrical stimulation differed from those prior to the sham electrical stimulation (ANOVA with repeated measures, $\alpha = 0.05$). The average firing rate did show a significant decrease after the sham electrical stimulation (ANOVA with repeated measures, $p = 0.022$). There was an overall trend for the average firing rate to decrease with time, likely an indication of a slowly degrading animal.

In contrast to the similarity of properties prior to electrical stimulation, the strong reduction in the peak t-score after electrical stimulation clearly stands out when comparing the receptive fields from both before and after electrical stimulation. As the t-score is normalized by the square of the number of spikes, this reduction likely is related to the reduction of this unit's firing rate from an average of 20.1 spikes per second before stimulation to 0.10 spikes per second after stimulation (as seen in Fig. 1H). On the other hand, the receptive field size does not noticeably change with electrical stimulation. When examining just the borders, presented in Fig. 1G, one sees that the extents of the receptive field prior to stimulation (light gray lines), immediately after electrical stimulation (black line), and around 6 h after electrical stimulation (darker gray lines) are almost identical. More to the point, there is no parametric statistical evidence (two sample t-test, $\alpha = 0.01$) or nonparametric statistical evidence (Wilcoxon rank sum test, $\alpha = 0.01$) that these trials do not share a similar mean size or median size. The rationale for a reduced confidence level is given below. Conversely, the reduction in the peak t-score values is statistically significant (one-tailed, two sample t-test, $p = 0.004$), as well as the reduction in average firing rate (one-tailed, two sample t-test, $p < 0.000$). Across the five units that were electrically stimulated, we observed a similar lack of significance in the changes in receptive field size with electrical stimulation when examining the units individually or as a group. On a unit-by-unit basis, the peak t-score and the average firing rate for this group exhibited both large increases and decreases but not necessarily always significant changes. The changes in peak t-score and average firing rate were not significant when combining the data for the all stimulated units.

Although significant changes in receptive field size were not seen at stimulated electrodes, significant changes in size were observed with units recorded on nonstimulated electrodes. Fig. 2 shows a representative sample of an unstimulated unit exhibiting a significant change in receptive field size with electrical stimulation. This unit is on an electrode neighboring the unit shown in Fig. 1 and the same format is used to display both the before and after electrical stimulation receptive field maps as well as the extracted receptive field properties. This unit's receptive field magnitude, borders, size, and firing rate are relatively consistent prior to stimulation. After stimulation, the border grows (black line encompassing the larger area in Fig. 2G) and the size becomes significantly larger (one-tailed, two sample t-test, $p = 0.004$), increasing from an average of 1.81 squared degrees to 2.41 squared degrees. However, the receptive field size appears to return to the prestimulation size in the trial presented in Fig. 2F, a measurement taken almost 6 h after termination of electrical stimulation. As in the case of the unit portrayed in Fig. 1, the magnitude of the peak t-score also becomes smaller after electrical stimulation but here the change is not significant (two sample t-test, $\alpha = 0.01$). Similarly, the average firing rate decreases with electrical stimulation but also is not significant (two sample t-test, $\alpha = 0.01$).

Interestingly, the added component of the receptive field that was observed in the first trial following electrical stimulation is contained within the receptive field of the neighboring electrically stimulated unit, which can be seen by comparing Figs. 1G and 2G. However, as this was the only case where receptive field data was available both for stimulated units and nearby units undergoing large receptive field size changes, this result can only be considered an interesting observation.

Although significant changes in receptive field size (and other receptive field properties) were observed for some units, the most of the units had nonsignificant changes in their receptive field properties. This led us to examine the relationship between electrode sites having significant changes in receptive field properties and sites of electrical stimulation. The false color plots of Fig. 3 show the magnitude and cortical-space distribution of the changes, with electrical stimulation, in

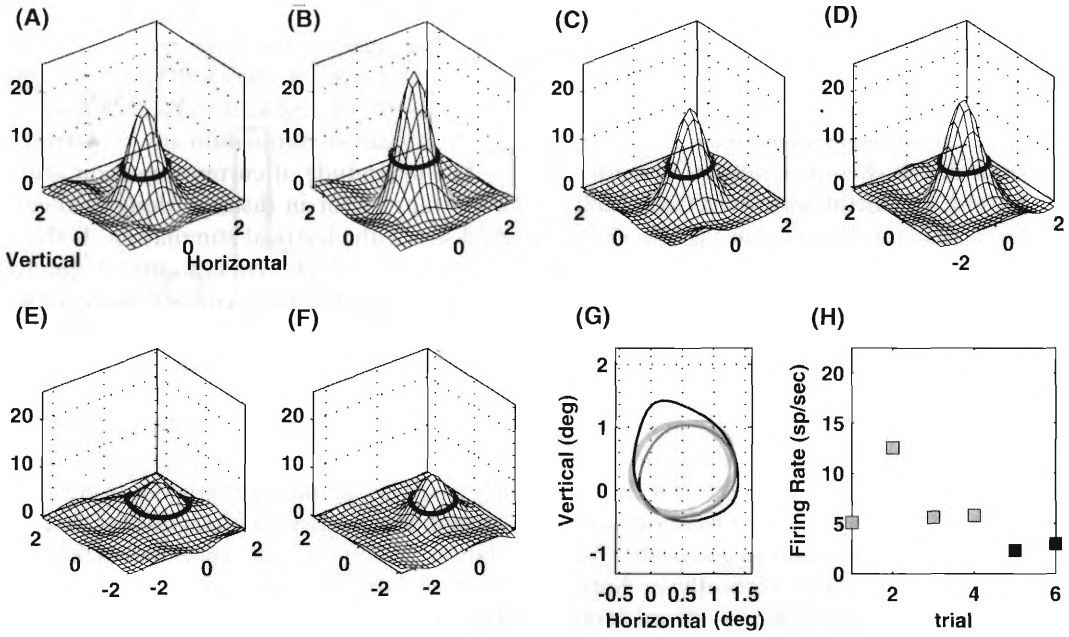

Fig. 2. Representative example of receptive field maps before and after electrical stimulation of a unit not on an electrically stimulated electrode but showing a significant receptive field size change. The panels of this figure are as described in Fig. 1. Here, the borders in panel G show that the first measurement taken after electrical stimulation (black line) is larger than those before stimulation are. Interestingly, the region of expansion is contained within the receptive field borders of the neighboring, electrically stimulated unit of Fig. 1.

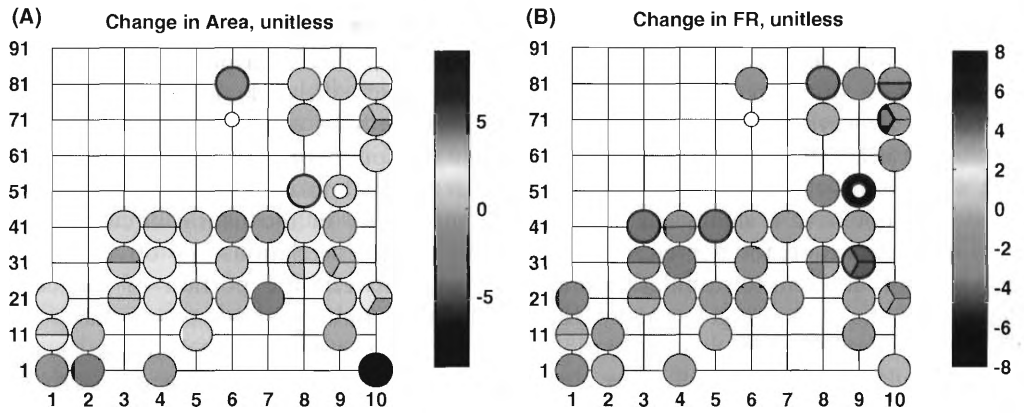

Fig. 3. Change in receptive field size (A) and firing rate (B) from a single animal as a function of position the unit was recorded on the electrode array. These data are shown on a 10×10 grid, representing the 10×10 grid of electrodes on the electrode array. Electrodes are numbered 1–100 with the bottom row representing electrodes 1–10 and the top row representing electrodes 91–100. For this particular animal, the array was implanted so that electrodes 1–10 are lateral and 1, 11, 21, etc. are rostral. At each electrode from which units were recorded, a circle is drawn. This circle is divided into a number of equal sized segments with one segment per unit extracted on the electrode. The interior of each segment of the circle is assigned a color depending upon the magnitude of the coefficient of variation, with the color bar to the right of the figure indicating the scale. If the change is found to be significant (two sample t-test, $\alpha = 0.01$ with the unnormalized data), then the border of the segment is colored red. Black borders indicate nonsignificant changes. A smaller, unfilled circle at the center of the electrode indicates that electrode was electrically stimulated. These data suggest that significant changes in receptive field size occurred around, but not at, stimulated sites and that that significant changes in responsiveness (firing rate) occurred at stimulated sites and, perhaps, in clusters away from stimulated sites.

receptive field size (Fig. 3A) and average firing rate (Fig. 3B) for the contralateral eye units in one animal. The change in each receptive field property is calculated as the value of the property after electrical stimulation less the average of the three measurements taken prior to electrical stimulation. Further, to highlight the significance of a change in magnitude of this difference, the data is normalized by dividing by the standard deviation of the three measurements taken prior to electrical stimulation. Hence, the difference data presented here is more correctly a coefficient of variation and is unitless. To reduce the possibility of a Type I error associated with multiple comparisons, a reduced significance level (0.01) was used to establish the significance of a change.

In this animal, the lone unit on a stimulated electrode where data are available (unit 59.01 on electrode 59, the unit presented in Fig. 1) exhibited a nonsignificant change in the receptive field size but a significant change in the firing rate. Across all of the experiments, none of the stimulated electrodes showed a significant receptive field size change. A unit on a neighboring electrode (unit 58.01 on electrode 58, the unit presented in Fig. 2) exhibits the exact opposite, a significant change in receptive field size without a significant change in the firing rate. Similarly, the unit on electrode 86, neighboring the stimulated electrode 76, exhibits a significant receptive field size change but not a significant firing rate difference. Across all animals, there was a tendency for significant increases in receptive field size to occur for units on electrodes that neighbored a stimulated electrode. Of the 18 significant receptive field size increases, 10 were on electrodes that were adjacent to a stimulated electrode. We defined adjacent electrodes as those vertically or horizontally adjacent, or 400 μm spacing, but not those diagonally adjacent, or 570 μm spacing. The remaining 8 units having a significant size increase were in a single animal. Three of these units were 570 μm from the nearest stimulation site, one was at 800 μm, two were at 1200 μm, one was at 1440 μm, and one was at 2040 μm. As a group, units adjacent to a stimulated electrode exhibited a larger proportion of significant receptive field size changes (19% with significant size changes) than units not adjacent to a stimulated site (4.3% with significant size changes). However, proximity to a stimulated electrode does not necessarily assure a significant change in size, as 43 units on electrodes adjacent to a stimulated electrode did not have a significant change in size. The empirical observation for a preference of large size increases for units adjacent to stimulation sites has statistical support. If the units are grouped by distance to the nearest stimulation site (at a stimulation site, adjacent to a stimulation site, and all other distances to a stimulation site), it was found that the adjacent group size grew by an average of 0.27 squared degrees whereas the other groups had nonsignificant size changes (ANOVA with repeated measures against the between-subjects grouping factor distance to nearest stimulation site, $p = 0.028$ with a posthoc Bonferroni comparison).

The distribution of changes in firing rate was not as clear. Typically, the units at stimulated sites had among the largest changes in firing rate. In some animals, units nearby the stimulation site showed significant decreases and, in some cases, the significant changes tended to cluster. However, statistical testing for clustering was not performed. Using the same grouping of distance given above, there was no statistical evidence that distance is an important factor in the changes in average firing rate (ANOVA with repeated measures against the between-subjects grouping factor distance to nearest stimulation site, $\alpha = 0.05$). The changes in peak t-score were also not significantly related to distance to the nearest stimulation site (ANOVA with repeated measures against the between-subjects grouping factor distance to nearest stimulation site, $\alpha = 0.05$).

The magnitude of current injection also appeared an important factor in distinguishing changes in receptive field size with electrical stimulation. If the animals were divided into two groups, low current stimulation current ($\leqslant 60\,\mu A$) and the high current stimulation ($\geqslant 150\,\mu A$), the changes in area were found to be significant with the low current group having increases in area and the high current group having decreases (ANOVA with repeated measures against the between-subjects grouping factor of current, $p = 0.001$). Similarly, the change in firing rate was significant with the firing rate of the high current group having a much greater decrease after stimulation (ANOVA with repeated measures against the between-subjects grouping factor of current, $p = 0.001$). The change in peak t-score did not significantly differ with current level (ANOVA with repeated measures against the between-subjects grouping factor of current, $\alpha = 0.05$).

3.3. Single-unit connectivity properties

Given the cortical-space distribution of the changes in the receptive field properties and the length of time over which these changes were induced, modification of strength of existing synapses is a likely candidate for the source of these changes. To investigate whether electrical stimulation affected the strength of synaptic connectivity in the neural circuit, we examined spike time cross-correlations. To obviate the need to distinguish between the portion of the correlated activity due to a common stimulus and that portion due to synaptic connectivity, we calculated the cross-correlation during periods of spontaneous activity. To separate the correlation due to direct synaptic connectivity from that due to global synchrony and other secondary effects (Eggermont & Smith, 1995), we examined the spike time cross-correlation of isolated spikes (Eggermont & Smith, 1996). Additionally, we found it advantageous to subtract out the mean cross-correlation across all delays between ±250 ms, making this statistic more properly termed the cross-covariance.

A representative example of the isolated spike cross-correlation between two units before and after electrical stimulation is shown in Fig. 4. Of the two units in this pair, neither was recorded at or near an electrode that was stimulated. The thin line is the cross-correlation before stimulation and the thick line is the cross-correlation after stimulation. The dashed lines are the 99% confidence interval for chance correlation, using the same thin and thick line representation. The cross-correlation as presented here is a histogram of the probability

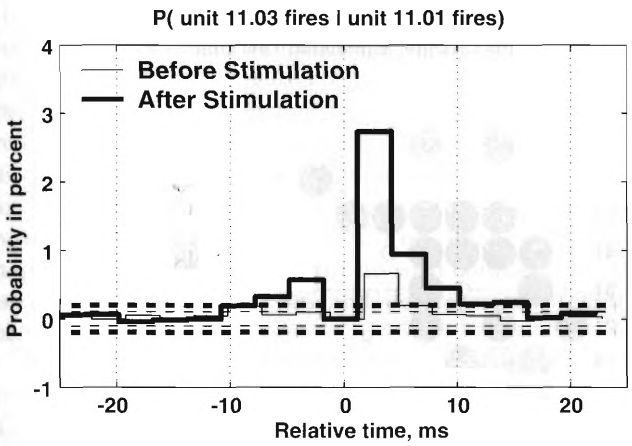

Fig. 4. Representative example of comparison of cross-correlation data before (thin line) and after (thick line) electrical stimulation. The thin and thick stippled lines indicate the 99% confidence levels for chance correlation. For this particular unit pairing, neither of which was electrically stimulated, the probability of the test unit (11.03) firing after the reference unit (11.01) was strengthened with electrical stimulation. The small probability of the reference unit firing after the test unit was also increased with electrical stimulation but remained small. An interpretation of these cross-correlations and changes is that the test unit is postsynaptic to the reference unit and that this connection was strengthened after electrical stimulation.

that the test unit (unit 11.03) fired at a time relative to the firing of the reference unit (unit 11.01), with relative time binned to 3 ms resolution. It should be noted that exchanging the roles of test and reference units would lead to both a reversal of the time axis and a change in the vertical scale. Two features of this cross-correlation stand out. First, the duration of significant correlation is within a ±10 ms window about zero, the same time period one might expect to observe effects of synaptic connectivity. Prior to using the isolated spike method, significant correlations were seen for up to 200 ms periods (data not shown), an indication of global synchronization (Eggermont & Smith, 1995). The second feature of this figure that stands out is that the cross-correlation data is not symmetric about zero relative time. For negative time lags, the correlation before electrical stimulation is not significant and is barely significant after stimulation. For positive time lags, the correlation is significant both before and after electrical stimulation. Further, the correlation becomes larger and longer lasting after electrical stimulation. In terms of the neural circuit, an interpretation of these cross-correlations and changes is that the test unit is postsynaptic to the reference unit and that this connection is stronger after electrical stimulation (Perkel, Gerstein, & Moore, 1967).

With the large number of units available, it is not practical to present all possible cases of unit pairings before and after electrical stimulation. Instead, we developed a scalar-valued metric of the degree of correlation for each unit pairing and compared this metric before and after electrical stimulation. To whit, the positive-time correlation was calculated as the average of the cross-correlation in the +1.5 to +10.5 ms time bins and the negative-time correlation was calculated as the average of the cross-correlation in the −1.5 to −10.5 ms time bins. The positive-time correlation represents the probability of the test unit firing in a time window following firing of the reference unit and the negative-time correlation represents the probability of the test unit firing before the reference unit. Separate metrics for positive-time and negative-time correlations were necessary, as most cross-correlations were not symmetric about zero relative time. For both correlations, the lower bound of 1.5 ms removes the potential confound of amplifier or electrode crosstalk. Across all possible cases, the metric of correlation ranged from −1.4% to 2.0%.

Using this metric of correlation, we examined the cortical space distribution of correlation changes with electrical stimulation. The false color plot of Fig. 5A displays the change in positive-time correlation at each test unit where the reference unit is a stimulated unit (electrode 62 marked with concentric unfilled circles). The data in this figure are shown in a similar format as Fig. 3 but without any interpretation of the significance of the change by the color of each segment's border. In addition, the interior color of a segment indicates a change in probability and has units of percent. A greater-than-zero change indicates the positive-time correlation was larger after electrical stimulation. For example, the unit on electrode 73 exhibited an increase (around 3.7%) in positive-time correlation with the unit on electrode 62 after electrical stimulation. This is indicated by the dark brown circle at electrode 73. Hence, the unit on electrode 73 had an increased probability, following electrical stimulation, of firing 1.5–10.5 ms after unit on electrode 62, where the unit on electrode 62 was most likely electrically stimulated. An interpretation of this result is that the unit on electrode 73 is postsynaptic to the stimulated unit and the synaptic strength increased with electrical stimulation. Analogous to the changes observed in receptive field size, most of the units showing large increases are in the neighborhood of the stimulation site. Further, there is a preference for increases over decreases. These results generalized to other stimulation sites and for other animals, an indication that electrical stimulation enhanced the strength of synapses with units postsynaptic to stimulated units, particularly for units near the stimulation site.

By reversing the roles of the test and reference unit, we can examine the changes in synaptic strength of units presynaptic to stimulated units. An example of these results is displayed in Fig. 5B, in which the change in positive-time correlation where the test unit is the stimulated unit on electrode 62 is displayed. There is a decrease in correlation with the stimulated unit

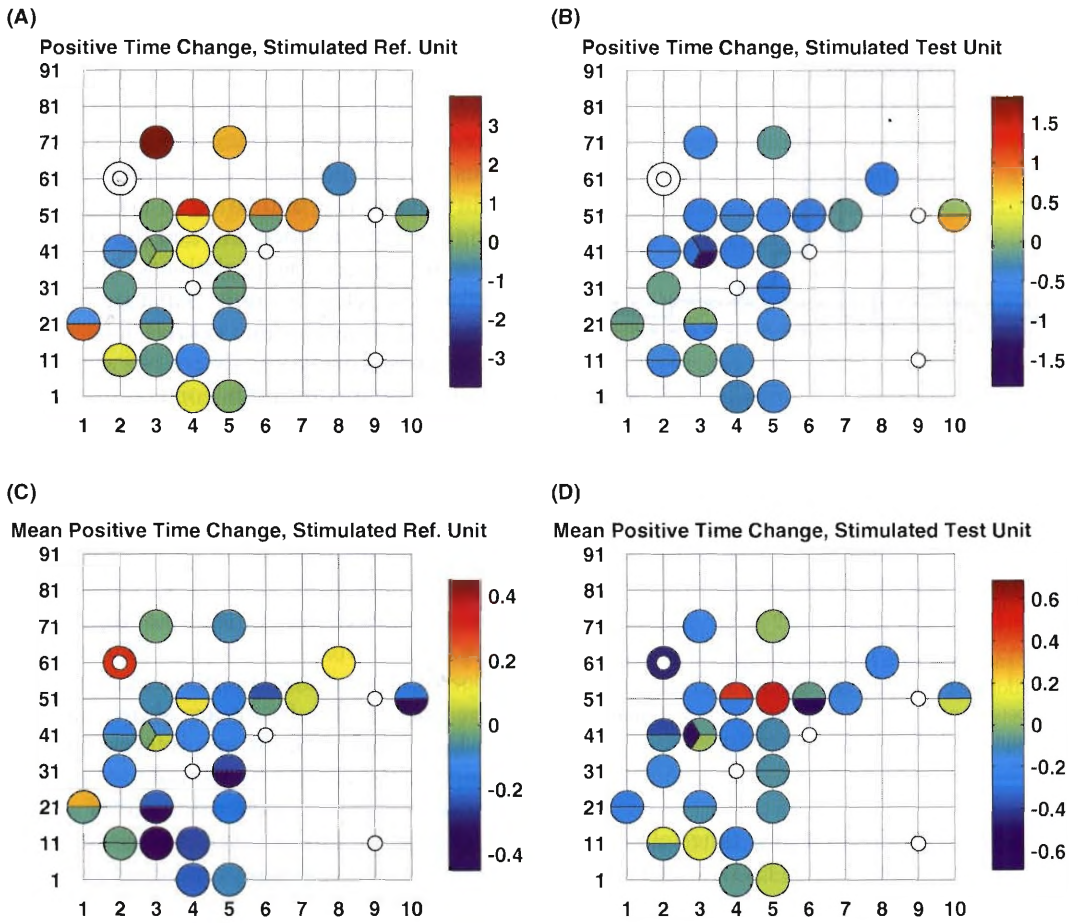

Fig. 5. Changes in correlation between before and after electrical stimulation from a single animal as a function of the position of the reference unit (A and C) or the test unit (B and D) was recorded on the electrode array. The magnitude of the correlation change is correlation after electrical stimulation is less than that before stimulation. Panel A shows the change in positive-time correlation as a function of test unit location where the reference unit is the unit recorded on electrode 62, a stimulated electrode, indicated by concentric unfilled circles. The largest increases are in the neighborhood of the stimulated site and in clusters separated from this site, indicating that these test units may be postsynaptic to the stimulated unit and their synaptic strength was increased with stimulation. Panel B shows the change in positive-time correlation as a function of reference unit location where the test unit is the unit recorded on electrode 62. The majority of the changes were decreases and without any pattern, indicating a wide spread reduction of synaptic strength when the stimulated unit is postsynaptic. In panel C, the average change in positive-time correlation is shown as a function of the position of the reference unit. The average is done across all test units except when the test and reference units were the same unit. The unit with the largest increase, the unit on electrode 62, is on a stimulated electrode, which indicates that the greatest increase in postsynaptic efficacy occurred, on average with the stimulated unit. In panel D, the average change in positive-time correlation is shown as a function of the position of the test unit. The average is done over all reference units except when the test and reference units were the same unit. The unit with the largest decrease is on a stimulated electrode, which indicates that the largest decrease in presynaptic efficacy occurred, on average with the stimulated unit.

throughout the array with only one unit pairing showing an increase. Similar results were found in the other animals and for other stimulation sites, indicating that electrical stimulation broadly reduced the strength of synapses with units presynaptic to stimulated units.

Even in this abbreviated format, it is not feasible to present all possible unit comparisons. However, if one averages the changes observed in Fig. 5A, the result gives an indication of the nature of the change for all test units against a particular reference unit, the unit on electrode 62 here. This process can be repeated with each unit acting as a reference unit to provide a single figure summarizing the extent of the changes. The false color plot of Fig. 5C shows the average change in positive-time correlation as a function of reference unit position on the array. That is to say, this figure illustrates the difference in positive-time correlation between after and before electrical stimulation averaged across all test units for each reference unit. (The autocorrelation data, where the test unit is the same unit as the reference unit, was not used in the average.) A large positive value indicates an increased probability of any other unit firing 1.5–10.5 ms after this particular reference unit following electrical stimulation. An interpretation of this result is

that the strength of postsynaptic connectivity with this reference unit, on average, increased after electrical stimulation. A large negative value indicates a decreased probability of any other unit firing 1.5–10.5 ms after the reference unit and such an outcome may be interpreted as the strength of the postsynaptic connectivity, on average, decreased. Among the potential interpretations of a near-zero change are that there was no change in postsynaptic connectivity strength (or no such connectivity exists) or that both increases and decreases in strength occurred and averaged out.

One feature of Fig. 5C that stands out is that an electrically stimulated electrode, electrode 62, has the largest average increase in positive-time correlation. That is, the unit on this particular electrode more strongly increased, on average, the strength of its synaptic connectivity with units postsynaptic to this unit when compared to units on electrodes that were not electrically stimulated. Across all experiments, the largest average increase in positive-time correlation was observed on electrically stimulated sites or their neighbors. However, the reverse was not necessarily true. That is, some stimulated sites showed little or no change in the average strength of positive-time correlation. This may be an indication that electrical stimulation was ineffectual in inducing firing of that particular unit.

The average change in positive-time correlation, as a function of test unit position, is shown in Fig. 5D. This figure illustrates the difference in positive-time correlation between after and before electrical stimulation averaged across all reference units for each test unit. Here, a large positive value indicates that for this particular test unit, the strength of its presynaptic connectivity, on average, increased and a large negative value indicates the strength of the presynaptic connectivity, on average, decreased. Again, stimulated sites (or their neighbors) stand out, having among the strongest decreases in negative-time correlation. Accordingly, the units on stimulated electrodes more strongly decreased, on average, the strength of synaptic connectivity with units presynaptic to this unit in comparison to units on electrodes that were not electrically stimulated. These same results were found in all animals tested. However, often units that were not near stimulation sites showed stronger decreases with stimulation. Together, Fig. 5C and D argue that electrical stimulation was effective at inducing changes in synaptic connectivity at or near stimulated sites.

A statistical examination of the change in positive-time correlation across all animals showed that units adjacent to a stimulation site had an increase in correlation and, further, the increase was significantly larger when stimulated with a high current level than when stimulated with a low current level (ANOVA with repeated measures against the between-subjects grouping factors of current, distance to nearest stimulation site and their interaction, $p = 0.021$). As more than one stimulation site maybe available as the reference unit for each correlation, the reference unit giving the large increase was used. The change in positive-time correlation did not significantly vary with current level alone (ANOVA with repeated measures against the between-subjects grouping factor of current, $\alpha = 0.05$) or distance to nearest stimulation site alone (ANOVA with repeated measures against the between-subjects grouping factor of distance to nearest stimulation site, $\alpha = 0.05$).

None of the animals showed any signs of the initiation of kindling, as indicated by after-charges in the local field potential, including the one with an electrical stimulation paradigm more conducive to kindling. We did see the appearance of what might have been after-discharges on a single electrode in a seventh animal but, due to manufacturing issues associated with the microelectrode array used in this animal, we are disinclined to make any statements about the initiation of kindling in this particular animal.

3.4. Correlation and receptive field properties changes

Given the result of large positive-time correlation increases for units near electrically stimulated units and the earlier result of receptive field size changes near electrically stimulated units, it is logical to propose a relationship between these two factors. In other words, if changes were to occur in the receptive field properties due to electrical stimulation, one might anticipate those changes occurring with units that exhibit large correlation changes.

However, if one examines only those units exhibiting a large positive-time correlation increase, where the reference unit is from an electrically stimulated electrode, there is no evidence to conclude that the poststimulation cases differ from the prestimulation cases (ANOVA with repeated measures, $\alpha = 0.05$ and the nonparametric equivalent, Friedman's test, $\alpha = 0.05$). This finding is found when treating each animal independently and when collecting all the animals together. If all units are separated into three groups; (1) electrically stimulated units, (2) units having a large positive-time correlation increase with a stimulated reference unit after electrically stimulation, and (3) all other units; there is no evidence to conclude that there is a significant interaction between the group assignment and trials taken before and after stimulation (ANOVA with repeated measures against the between-subjects factor of the grouping, $\alpha = 0.05$). A large positive-time correlation increase was deemed to be an increase of 1.0% or greater, out of an observed range of correlation changes of −1.3% to 3.7% (Dragoi et al., 2001; Pettet & Gilbert, 1992; Schuett et al., 2001; Spengler & Dinse, 1994), resulting in 29 units having large positive-time correlation increases. Neither more conservative nor more

liberal definitions of what comprised a large positive-time correlation increase changed the result. Further, if one considers the positive-time correlation change with a stimulated reference unit after electrically stimulation to be a covariate, there is no evidence that the magnitude of the receptive field size change is linearly related to the change in correlation (ANOVA with repeated measures against the covariate correlation change, $\alpha = 0.05$). Similar nonsignificant findings occurred when examining changes in peak t-score and firing rate.

The lack of relationship in the receptive field size change with stimulation and with degree of correlation change can be seen in the error bar plots of Fig. 6. Here, the mean size for all units is plotted as a function of trial number relative to electrical stimulation for each of the groups described in the previous paragraph. Only three trials prior and two trials after electrical stimulation are shown to assure nearly equal size samples for all trials. To remove the portion of the variance in the mean size associated with different units having different receptive field sizes, the mean size across all five trials was subtracted out on a per unit basis before generating the group means and their confidence intervals. Although the units with large positive-time correlation increases tend to show an increased size for the first trial after stimulation and the stimulated units appear to have a decreased size after stimulation, this trend is not significant as indicated by the width of the confidence intervals.

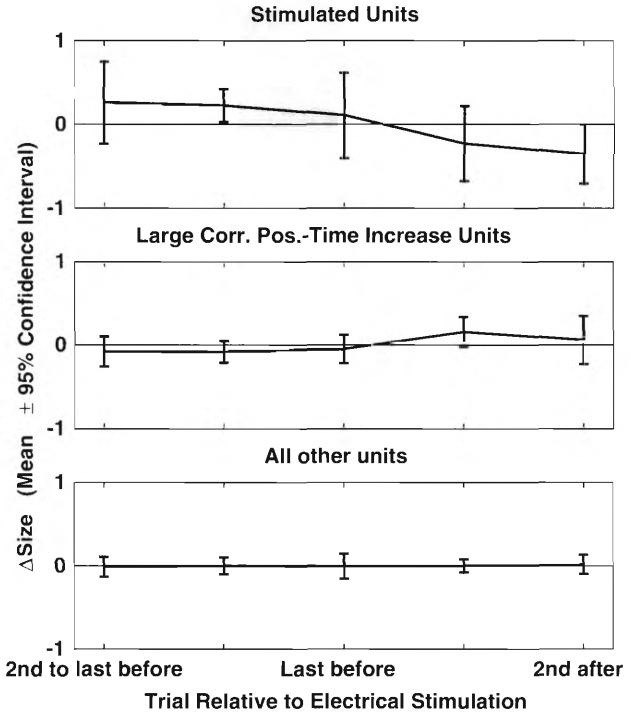

Fig. 6. Comparison of the change in receptive field size data shows that the difference between the three trials before and the two trials after electrical stimulation is within the variability of the measurement. The units have been divided into three groups, units on stimulated electrodes, units showing a large increase (>1.0%) in positive-correlation with a unit on a stimulated electrode, and all other units. The group of units on stimulated electrodes appears to have a trend of a decreasing size following electrical stimulation. In contrast, the group of units showing a large increase in positive-correlation appears to have an increased size immediately following electrical stimulation. However, neither of these trends is significant as indicated by the confidence intervals not precluding a zero mean.

Even if the size of a unit's receptive field does not change with electrical stimulation, it is possible that the subregions of the receptive field could change their responsiveness to visual stimulation. Reorganization within the subregions would be manifested as a movement of the visual space location of the receptive field, calculated as the center of mass of the t-score statistics within the receptive field. However, a distinction must be made between movement associated with inevitable eye drift occurring over the duration of an experiment and movement associated with receptive field reorganization. To separate the two types of movement, the group of 'all other units' described above was assumed to only undergo eye drift movements. Using this set of units, all measurements of the receptive field location were optimally, in a least-mean-square sense, rotated and translated to coregister with the last location measurement taken prior to electrical stimulation. With all of the measurements of receptive field location coregistered, the change in the receptive field's location consists of measurement error and movements associated with receptive field reorganization. After performing this procedure, we examined the distribution of receptive field location changes, with electrical stimulation, for the group of units having a large positive-time correlation increase with a stimulated reference unit after electrical stimulation. Although the change with electrical stimulation of the receptive field location for this group was widely dispersed, there is no evidence to conclude that the distribution of these measurements is not due to the variability in the measurement (Kolmogorov–Smirnov test comparing the distribution of the length of the location change vector for the groups 'units having a large positive-time correlation increase with a stimulated reference unit after electrically stimulation' and 'all other units', $\alpha = 0.05$).

4. Discussion

In this manuscript, we have described our findings on the changes observed in the functional organization and synaptic connectivity in primary visual cortex that arise from electrical stimulation, primarily with an eye towards the impact these changes would have on a cortically-based neuroprosthesis. We found that the receptive field size of neurons in the neighborhood of stimulation sites could increase with electrical

stimulation and the same group of neurons could undergo increased synaptic efficacy with electrical stimulation. Although these changes were statistically significant, the magnitude of these changes was minimal and likely would not greatly affect the development and use of a cortically based vision neuroprosthesis.

Specifically, we found that the receptive field size significantly changed for some units, particularly those near sites of electrical stimulation. In the one case where adequate data was available, the increased receptive field size of a unit nearby to stimulation site was brought about by this unit taking on some of the receptive field location of the stimulated unit. None of the units at electrically stimulated electrodes demonstrated a significant receptive field size change. The stimulating current magnitude was also found to affect significantly the nature of the receptive field size change. At low current levels ($\leqslant 60\,\mu A$), the size tended to increase and at high current levels ($\geqslant 150\,\mu A$), the size tended to decrease. The reduction in size with high current may be a sign of either neural exhaustion or, more likely, tissue damage. Using an estimated electrode tip surface area of 1573 squared microns (Rousche & Normann, 1999), a current level of $150\,\mu A$ results in a charge density of $1900\,\mu C/cm^2$, well above the charge density where irreversible chemical reactions to are initiated ($75\,\mu C/cm^2$) and potential tissue damage occurs (Robblee & Rose, 1990).

Furthermore, the responsiveness to visual stimulation, parameterized by the average firing rate during visual stimulation, exhibited significant differences between before and after electrical stimulation, with a reduction in the average firing rate after stimulation. As with the size change, responsiveness changes were observed near stimulation sites but unlike the situation for size changes, significant responsiveness changes were observed at stimulation sites. Larger current magnitudes tended to cause a larger reduction in firing rates than lower current magnitudes, likely a sign that the higher currents damaged the neural tissue. However, care must be taken with changes in average firing rates as we observed significantly large changes in this property prior to initiation of electrical stimulation. The nature and cortical distribution of changes in receptive field properties are very similar to those observed in rat auditory cortex after electrical stimulation (Maldonado & Gerstein, 1996b).

We also observed a possible substrate for the receptive field size changes following electrical stimulation, an apparent increase, with electrical stimulation, in synaptic strength for units postsynaptic to stimulated units. This increase was inferred from the observation that some units had an increased probability of firing in a 10 ms window following the firing of a stimulated unit. Although changes in probability of temporally related activity were observed for many unit pairings, there was a preference for increases in probability when the stimulated unit fired first. The largest average increase in probability of temporally related activity with stimulated units tended to occur at or near the site of stimulation and the higher current intensity tended to cause larger increases. Despite the changes in synaptic strength, we saw no clear signs of kindling. Again, our results show a similar nature and distribution as those results observed after electrical stimulation of rat auditory cortex (Maldonado & Gerstein, 1996a).

Despite evidence of clustering of both the changes in receptive field size and the increase in synaptic strength in the neighborhood of stimulated units, we were unable to extract a significant relationship between these two factors. Neither segregating units by level of positive-time correlation with stimulated units nor using the degree of correlation with stimulated units as a covariate led to a meaningful change in the measurement of receptive field size with electrical stimulation. This lack of a significant relationship between changes in receptive field size and positive-time correlation with stimulation is likely the result of the subtlety of the changes in both the receptive field size and correlation. The change in receptive field size with electrical stimulation was rather modest, representing only a small portion of the average receptive field size prior to stimulation. The majority of size changes were less than $\pm 12\%$ of the size prior to stimulation. The change in positive-time correlation was also small, being limited to the range of -1.3% to 3.7%. This implies that there was only, at best, an average of one additional firing of the postsynaptic unit for every 25 spikes observed at the reference unit. Given the small magnitude of the changes for each factor, it is quite possible that their interaction effect would be too small to be significant.

In a recent report (Schuett et al., 2001) it has been suggested that synaptic strength can be both increased and decreased depending on the temporal relationships between the presynaptic and postsynaptic activity, an experimental finding that concurs with Hebb's postulate (Hebb, 1949). In those experiments, a precise temporal relationship of synaptic activity was introduced by electrically stimulating at times relative to the introduction of visual stimulation. In our experiments, the electrical stimulus was applied nearly continuously and in the absence of any visual stimulus. Nevertheless, one might anticipate changes in connectivity strength arising from the naturally occurring spontaneous activity of the neurons not electrically stimulated. However, as the relative timing of this spontaneous activity and the electrically induced activity is random, we might expect both increases and decreases in synaptic strength.

The expected effects of electrical stimulation can readily be understood by the simple model presented in Fig. 7. Although this model greatly simplifies the thalamocortical and corticocortical synaptic connectivity in cortex and ignores the recurrent loops and the possibility of intermediary neurons within the chain, it captures

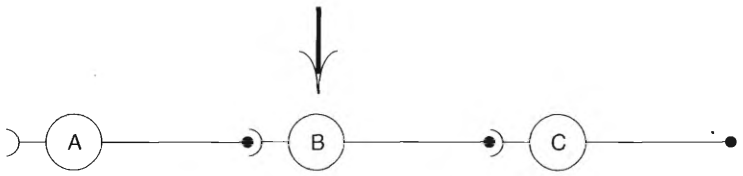

Fig. 7. Model of expected reorganizational effect of electrical stimulation. One would expect an increase in synaptic efficacy with neurons postsynaptic (C) to the stimulated neuron (B) and a decrease in synaptic efficacy with neurons presynaptic (A) to the stimulated neuron.

the essence of the connectivity. In this model of three neurons, the electrically stimulated neuron (neuron B) is postsynaptic to neuron A and presynaptic to neuron C. No presumption of the location of neuron A is inferred by the model; neuron A may be thalamic or the entire circuit may reside in striate cortex. Neuron B is more active, due to electrical stimulation, than neurons A and C, which only fire randomly having no visual input. When neuron C fires, it is likely that neuron B also fired and, by Hebbian learning, this synaptic connection will be strengthened. In contrast, when the stimulated neuron fired, it is unlikely that neuron A spontaneously fired. Hence, by the corollary to Hebbian learning, neurons that do not fire together do not wire together, this synaptic connection is expected to be weakened.

The perceptual impacts of these synaptic changes are also straightforward. As the BC synapse is strengthened, one might anticipate that neuron C will take on more of the characteristics of the electrically stimulated neuron (neuron B) such as its location in visual space. This may either lead to an increase in receptive field size, if C's receptive field was not already contained in B's receptive field, or a shift in C's receptive field location more toward the location of neuron B. The exact opposite would occur with the relationship between neurons A and B. That is, neuron B would take on less of the characteristics of neuron A.

Much of our results match these expectations. Neurons in the neighborhood of a stimulated neuron, presumably neuron C in the model, increased their synaptic connectivity with the stimulated neuron and their receptive field size increased. In the one case where we have the necessary data, a neighboring neuron took on some of the visual space representation of a stimulated neuron. We also saw a broadly distributed reduction in the synaptic connectivity of neurons presynaptic to the stimulated neuron. We did not see any reduction in receptive field similarity but, often, such results are difficult to show.

A similar understanding can be had of the impact that reorganization will have on a visual neuroprosthesis, a primary driver of this research. In most cases of blindness, some of the neurons of the subcortical visual pathway are spared and fire randomly. Further, in the blind, the neurons of striate cortex firing randomly. Hence, both the connectivity changes and "perceptual" changes described above apply in the blind. A strengthening of the B–C synapse will lead to a stronger likelihood that neuron C will fire subsequent to electrically inducing activity in neuron B. Hence, the induced phosphene will acquire more of C's characteristics than before stimulation. Given that neuron B is presynaptic of thousands of other neurons, this leads to a concern that the induced phosphene might grow to encompass an enormous region of visual space. However, the small changes in receptive field size that we observed indicate this concern is unfounded. Our data suggests some increase will occur but only over a limited extent and that these changes might be mitigated by the more extensive, but more randomly distributed, stimulation that will occur in a visual neuroprosthesis. The perceptual impact of the reduction in strength in the A–B synapse is unclear as the normal synaptic input is not the foremost drive of the stimulated neuron in a prosthetic application. This raises the interesting intellectual question of the impact random peripheral activity will have on a clinical visual neuroprosthesis. One might anticipate that this input will appear as visual noise, but to date no experimental subjects have reported such (Bak et al., 1990; Brindley & Lewin, 1968b; Dobelle & Mladejovsky, 1974).

Despite the clear evidence of changes, care must be taken in interpreting these results as there were had from anesthetized animals over a short period of time. The minimal changes in receptive field size and positive-time correlation potentially could be a result of anesthetic reducing the potential for plastic changes. Although the ability to induce plasticity under anesthesia is well established (Dragoi et al., 2001; Pettet & Gilbert, 1992; Schuett et al., 2001; Spengler & Dinse, 1994), the impact of the anesthetic agents on the degree of plastic changes was not the focus of this study. Further, the observed effects may be the result of electrical stimulation causing localized tissue damage, not neural organization. Although a technically challenging task, we look forward to repeating these experiments in awake, behaving animals over longer periods.

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