Somatosensory & Motor Research 1998; 15(2):146–156

CARFAX

Laminar differences in bicuculline methiodide's effects on cortical neurons in the rat whisker/barrel system

HAROLD KYRIAZI[1], GEORGE E. CARVELL[2], JOSHUA C. BRUMBERG[1] and DANIEL J. SIMONS[1]

[1]*Department of Neurobiology, School of Medicine,* [2]*Department of Physical Therapy, School of Health and Rehabilitation Sciences, University of Pittsburgh, Pittsburgh, PA 15261, USA*

Abstract

Extracellular unit recordings were made at various depths within SmI barrel cortex of immobilized, sedated rats, in the presence and absence of titrated amounts of the $GABA_A$ receptor antagonist bicuculline methiodide (BMI). Principal and adjacent whiskers were moved singly, or in paired combination in a condition-test paradigm, to assess excitatory and inhibitory receptive field (RF) characteristics. Neurons were classified as regular- or fast-spike units, and divided into three laminar groups: supragranular, granular (barrel), and infragranular. BMI increased response magnitude and duration, but did not affect response latencies. The excitatory RFs of barrel units, which are the most tightly focused on the principal whisker, were the most greatly defocused by BMI; infragranular units were least affected. All three layers had approximately equal amounts of adjacent whisker-evoked, surround inhibition, but BMI counteracted this inhibition substantially in barrel units and less so in infragranular units. The effects of BMI were most consistent in the barrel; more heterogeneity was found in the non-granular layers. These lamina-dependent effects of BMI are consistent with the idea that between-whisker inhibition is generated mostly within individual layer IV barrels as a result of the rapid engagement of strong, local inhibitory circuitry, and is subsequently embedded in layer IV's output to non-layer IV neurons. The latter's surround inhibition is thus relatively resistant to antagonism by locally applied BMI. The greater heterogeneity of non-granular units in terms of RF properties and the effects of BMI is consistent with other findings demonstrating that neighboring neurons in these layers may participate in different local circuits.

Key words: *somatosensory, thalamocortical, microiontophoresis, GABA, cortical column, receptive field*

Introduction

Studies of rodent primary somatosensory SMI cortex have consistently revealed laminar differences in functional organization. Properties that differ with cortical depth include response timing and latency (Carvell and Simons, 1988; Agmon and Connors, 1992; Armstrong-James *et al.*, 1992; Alloway *et al.*, 1993), modality specificity (Lamour *et al.*, 1983; Dykes and Lamour, 1988), thalamocortical connection efficacy (Johnson and Alloway, 1996), excitatory receptive field (RF) size (Simons, 1978; Simons and Woolsey, 1979; Lamour *et al.*, 1983; Ito, 1985; Chapin, 1986; Simons *et al.*, 1992), spontaneous activity (Dykes and Lamour, 1988), the effect of acetylcholine on RF properties (Lamour *et al.*, 1988), and the organization of surround inhibition (Simons, 1985). Moreover, the RF properties of layer IV neurons are considerably more homogeneous than those in other laminae (Simons, 1978, 1985; Chapin, 1986; Brumberg, 1997). As in other species and cortical areas, neurons in different layers display characteristic morphologies, receive different patterns of extrinsic and intrinsic inputs, and are likely to participate in different local circuits (see White, (1989)). A distinctive feature of the somatosensory cortex in rodents is the presence of readily identifiable collections of layer IV neurons, called barrels, that relate one-to-one to individual whiskers on the contralateral face (Woolsey and Van der Loos, 1970; Welker, 1976). Because of the punctate organization of both the sensory periphery and its cortical representation, it has been relatively easy to ascribe some of the laminar dependent functional properties of barrel cortex neurons to interlaminar and intercolumnar connections (for review, see Armstrong-James (1995) and Simons (1995)).

We have examined in detail the operations performed by local circuitry within individual layer IV barrels (Simons and Carvell, 1989; Kyriazi and Simons, 1993). More recently, we utilized microiontophoretic applications of γ-amino butyric acid (GABA) and some of its pharmacological agonists and antagonists and found that a range of response properties could be influenced by the drugs (Kyriazi

Correspondence: Harold Kyriazi, Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA. Tel: (412) 648-9443; Fax: (412) 648-1441; E-mail: htk + @pitt.edu.

0899-0220/98/020146–11 $9.00

et al., 1996a,b). A consistent finding was that GABA and its agonists disproportionately diminished, and its antagonists disproportionately enhanced, responses that were initially smaller. We proposed that these non-linear effects were due to non-selective, drug-induced shifts in resting membrane potential (Kyriazi *et al.*, 1996a). Given that clear differences exist in the RF properties of neurons in other layers, we wanted to examine whether they were affected similarly enough to support the idea that GABA acts by the same non-selective mechanism across cell types and different local circuits. Here we compare excitatory and inhibitory RF properties of neurons in the barrel with those in other layers, in the presence and absence of the $GABA_A$ receptor antagonist bicuculline methiodide (BMI). The findings demonstrate that qualitatively similar non-linear effects of BMI are observed throughout the cortical column, suggesting a common mechanism for GABA's influence on RF properties. Further, the data suggest that the inhibitory surrounds of many supra- and infragranular neurons are built into their excitatory inputs, having originally been generated by layer IV circuitry. Non-granular neurons differed widely in the extent of BMI-induced reduction of surround inhibition, consistent with the possibility that even neighboring neurons in these layers participate in different types of local circuitry.

Methods

Animals

Experiments were conducted on 17 female, 200–350 g Sprague-Dawley rats. Surgical procedures were as previously described (Simons and Carvell, 1989; Kyriazi *et al.*, 1996a). Briefly, under halothane anesthesia, a tracheotomy and femoral artery and external jugular vein catheterizations were performed. A steel post was affixed to the skull with dental acrylic, and used to hold the animal's head. A small craniotomy was made over the whisker representation of the right primary somatosensory cortex, an acrylic dam was constructed around it to help keep the pial surface moist, and an incision was made in the dura. Upon completion of all surgery, halothane was discontinued and the animal was sedated by continuous intravenous (i.v.) infusion of fentanyl, a synthetic opiate (Sublimaze, *Janssen Pharmaceuticals*; 5–10 μg/kg/h), immobilized with pancuronium bromide (~1.6 mg/kg/h i.v.), warmed via a servo-controlled heating blanket (Harvard Apparatus), and artificially respired with a humidified mixture of oxygen and nitrogen (33–50% O_2). Ophthalmic ointment was applied to prevent corneal drying. The rat's physiological condition was assessed by monitoring mean arterial blood pressure, electroencephalogram, glabrous skin perfusion, and pupillary reflexes. The airway pressure waveform was also displayed to monitor tracheal patency.

At the conclusion of an experiment, or if normative physiological conditions could not be maintained, the animals were administered a lethal dose of pentobarbital sodium i.v., and perfused transcardially (see Kyriazi *et al.* (1994)). Tangential sections of flattened, frozen cortex were cut (30 or 60 μm), and alternate sections were processed for either cytochrome oxidase or horseradish peroxidase (HRP) histochemistry along with a Nissl counterstain. Sections were later examined (see below) to reconstruct electrode tracks, which were marked with small HRP deposits (see Simons and Land (1987)).

Whisker stimulation

Preliminary multi-unit mapping of the SmI cortical whisker representation was performed using tungsten microelectrodes and hand-held whisker stimulators. For obtaining quantitative data about single-unit response properties, whiskers on the left-hand side of the face were deflected singly or in paired combination by piezoelectric stimulators (Simons, 1983) that contacted the whiskers ~10 mm from the face. Data collection was controlled by an LSI 11/73 computer as previously described (Simons and Carvell, 1989; Kyriazi *et al.*, 1996a).

Assessment of excitatory RFs. A neuron's principal whisker (PW) is defined physiologically as that whisker whose movement yields the largest response, and which, invariably for barrel neurons, upon histological examination, also corresponds to that barrel's somatotopically represented whisker. PWs or adjacent whiskers (AWs) were deflected, one at a time, randomly in each of eight different directions (in 45° increments relative to the horizontal alignment of the whisker row). Ramp-and-hold deflections were 1 mm in amplitude, 200 ms in duration, and had onset and offset velocities of ~125 m/s. Randomized sequences of the eight deflections were delivered ten times for a total of 80 stimuli. Interstimulus intervals were 1.5 s. The strengths of PW and AW responses were quantified using responses (spikes/stimulus) to stimulus onsets averaged over all eight deflection angles.

A measure of the relative size of the AW response, called *'RF focus'*, was defined as the ratio of the AW response to stimulus onset (ON response) averaged over all eight deflection angles to that of the PW (Kyriazi *et al.*, 1996b). A value near zero indicates a RF tightly focused on the PW, while a value near 1.0 indicates a broad focus, where the AW drives the cell nearly as well as the PW.

Assessment of the inhibitory RFs. AWs and PWs were deflected, in that order, with a 30 ms separation, in a 'condition-test' paradigm. The conditioning

stimulus (AW movement) was delivered at each of eight angles, while the test stimulus (PW deflection) was delivered at the unit's maximally effective deflection angle. The test whisker was also deflected alone, at its best angle, to establish a baseline response. A battery consisted of ten sets of eight paired stimuli, along with ten test-alone stimuli, for a total of 90 stimuli. The ratio of conditioned-test to test-alone response is termed the *condition-test ratio* (CT ratio), and is used as an inverse measure of the strength of surround inhibition. A completely inhibited response would have a value of 0; a completely uninhibited response would have a value of 1.0.

Electrophysiological recordings and microiontophoresis

Multi-barrel microelectrodes were prepared as described previously (Kyriazi *et al.*, 1996a). A carbon fiber, 7 μm in diameter, was loaded into the central barrel of a seven barrel glass capillary tube assembly. After the glass assembly was heated and pulled to a tip diameter of ~ 12 μm, the carbon was etched electrochemically to a pencil-point shape and then positioned to protrude from the glass tip by 5–10 μm. In addition to the carbon fiber in the central barrel, two barrels contained BMI (2.5 mM in 0.9% NaCl), two contained GABA (0.2 M, pH adjusted to 3.0 with HCl), one saline (0.9% NaCl) for current balancing, and one HRP (10% in 50 mM Tris–HCl buffer, pH 6.8) for marking electrode tracks. Iontophoretic barrels had resistances of ~ 22 MΩ. Holding currents were -15 nA. The electrode was advanced perpendicularly through the cortical tissue using a hydraulic microdrive equipped with a stepping motor and digital counter.

In initial experiments, BMI currents were titrated by ejecting a GABA current sufficient to cause an approximate 50% reduction in PW ON responses at the maximally responsive deflection angle, and then providing sufficient BMI current to return activity to baseline, after which the GABA current was removed. This usually resulted in an approximate two-fold increase in the PW ON response. For later experiments, titration with GABA was dispensed with, and the BMI current was adjusted to cause an approximate two-fold increase in PW maximal angle ON response, assessed by a whisker stimulus program that provided 'running boxcar' averages of activity over the previous ten stimuli. Average BMI currents (mean $\pm$ standard deviation (SD)) were 5.8 ± 5.9 nA, 8.4 ± 5.3 nA, and 9.6 ± 6.5 nA, respectively, for supragranular, middle, and infragranular regular-spike units (differences not significant).

Spike train data, in the form of sequential interspike intervals (100 μs resolution), were obtained from extracellular recordings of single units isolated by amplitude discrimination, and were used for on-line peristimulus time histogram (PSTH) construction and off-line data analysis. Regular- and fast-spike units were distinguished on the basis of the timecourse of their action potentials. As previously described (Kyriazi *et al.*, 1996a), neurons judged to be fast-spike units were found to have products of 10–90% risetime and peak width at half height (of the main, positive-going peak) < 0.025 ms^2, while units judged to be regular-spike units had larger products.

Layer designations

Cortical neurons were divided into three categories—supragranular, middle and infragranular—based on microdrive depth readings and their correspondence with cytochrome oxidase and Nissl staining. We consider these designations to correspond to layer II/upper layer III, lower layer III/layer IV, and layers V–VI. There are likely to be misassignments of neurons at the borders. This is due to the spatial resolution of the microelectrode recording and because the thickness of the cortical barrel decreases somewhat with distance from its horizontal center (see Figure 1B of Akhtar and Land (1991)). We set the zero point of the microdrive when the electrode tip touched the thin layer of saline on the brain surface rather than when it actually penetrated the pia mater; therefore our nominal depths are ~ 50–150 μm too great (e.g., see Figure 9 of Simons (1978)). Given this bias in our microdrive depth readings, which has been consistently confirmed on the basis of HRP marker spots, our population of middle layer units likely encompasses neurons whose somata lie 600–900 μm below the pial surface. For the present analyses, the designation of 'middle layer' is further restricted in that only barrel neurons are included. The terms middle/barrel, upper/supragranular, and lower/infragranular will be used interchangeably.

Data analysis

ON responses were measured over 20 ms intervals, a period long enough to encompass the entire response. Spontaneous activity was measured in a 100 ms interval immediately preceding stimulus onset. Spike train data were processed using customized FORTRAN programs, and statistical analyses were performed using a PC-based statistical package (SPSSPC+, SPSS). Two-tailed p values less than 0.05 were taken to be significant. Unless otherwise indicated, data are presented as the mean $\pm$ SD.

As one measure of response timing, we calculated '*modal latencies*' as follows. Beginning 5 ms after the initiation of whisker movement, the time of the first spike in the 20 ms response window was taken for each of 80 trials, at 0.5 ms resolution. The bin with the greatest number of first spikes was taken as the mode. For five of the units analyzed, there were no

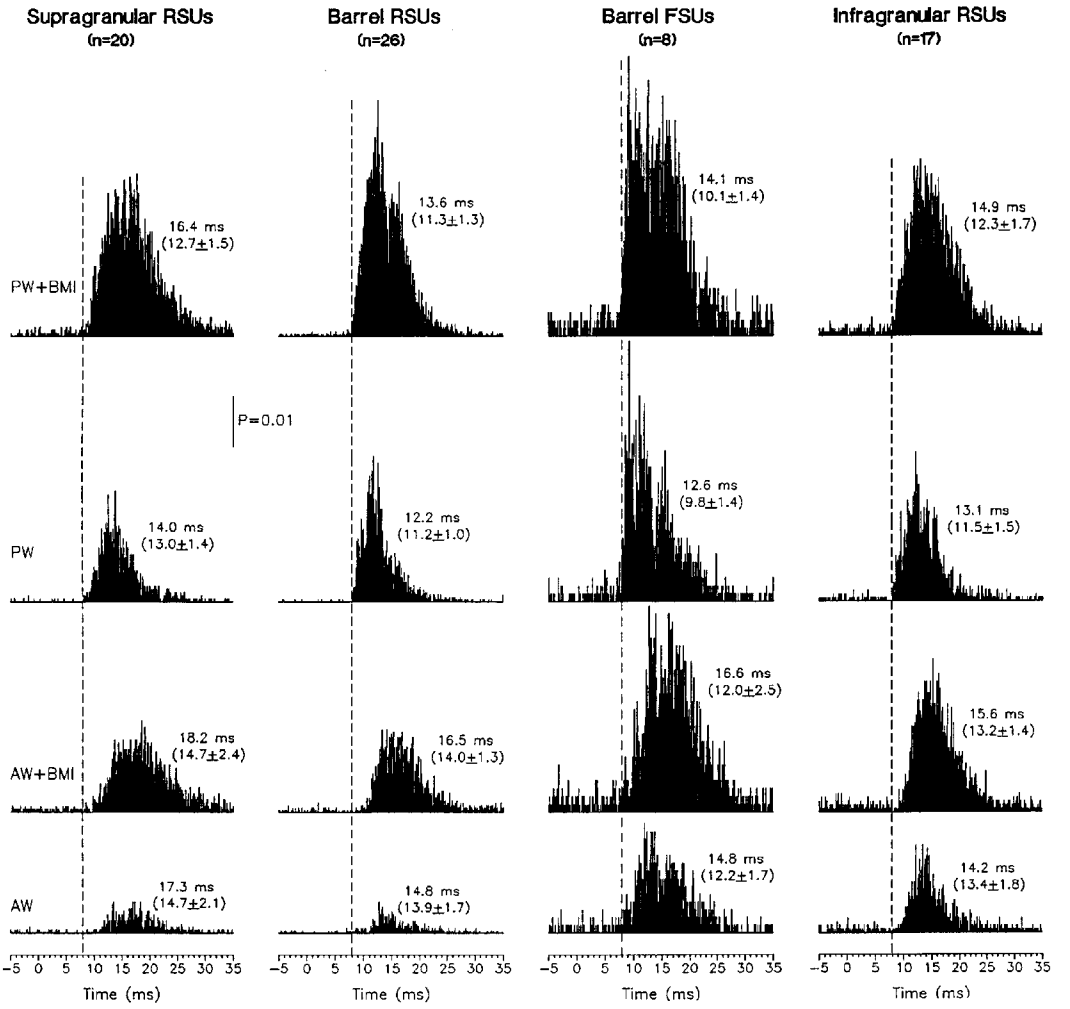

FIGURE 1. Population peristimulus time histograms (PSTHs) of ON responses to principal and adjacent whisker movement. Whiskers were deflected at eight different angles a total of ten times each, and *n* values refer to the number of units that comprise each population. Four different populations are depicted, each under four conditions, as labeled at the left. The scale bar applies throughout, and indicates the probability of a spike/100 μs bin/whisk unit. Numbers indicate the time of the population median spike; numbers in parentheses indicate mean modal latencies, ± standard deviation. Dotted vertical lines indicate the 8th ms after stimulus onset, for comparison of the times the various responses begin. AW, adjacent whisker; BMI, bicuculline methiodide; FSU, fast-spike unit; PW, principal whisker; RSU, regular-spike unit.

bins with more than one spike, and these were rerun using 1.0 ms bins. If in this case there were still no bins with more than one spike, the unit was excluded from the analysis (three units were excluded from the latency analyses on this basis).

Results

Data are reported from 63 regular-spike units and 14 fast-spike units obtained in 28 vertical electrode penetrations from 17 experiments.

BMI's effects on excitatory response timing

BMI enlarges and prolongs the responses to discrete whisker movements, but does not alter response latencies. Figure 1 shows population PSTHs of the PW and AW ON responses of supragranular, barrel and infragranular regular-spike units, and barrel fast-spike units, in the presence and absence of BMI. BMI clearly increased response magnitude, and the responses were longer lasting, i.e., the populations' median spikes occur later with BMI present (unparenthesized numbers). Response onset times are generally unaffected by BMI, as judged both by the leading edges of the population PSTHs and by the individual units' modal latencies (numbers in parentheses). In one case, that of the infragranular PW response, BMI caused a slight (0.8 ms) but significant ($p = 0.018$, pair t-test) increase in the modal latency.

Figure 1 also shows that AW responses occur later than PW responses, both with and without BMI. In all cases, the populations' median spikes and modal latencies of individual units' responses occur ~1–3 ms later than those of the PW; all modal latencies differed significantly between PW and AW deflections in pair t-tests (except for the supragranular regular-spike units in the presence of BMI, in which case the p value was 0.057).

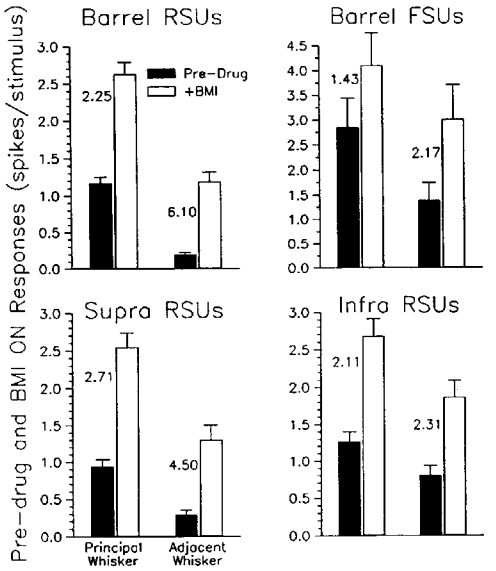

FIGURE 2. Effect of bicuculline methiodide (BMI) on principal and adjacent whisker ON response magnitudes. The same peristimulus time histogram (PSTH) data depicted in Figure 1 are here quantified for the four populations. Numbers indicate the proportional increase caused by BMI. Note that for the barrel and supragranular regular-spike units (RSUs), BMI causes a much larger proportional increase in adjacent whisker responses than in principal whisker responses. The same n values apply as in Figure 1. Error bars indicate standard error. FSU, fast-spike unit.

BMI's effects on excitatory response magnitude

Figure 2 quantifies the ON response PSTHs shown in Figure 1, and illustrates two additional points. One is that in superficial and middle layer neurons, BMI causes much larger proportional increases in AW ON responses than in PW responses. In the case of barrel regular-spike units, for example, the average AW ON response increased ~six-fold, whereas PW responses approximately doubled. These data are consistent with our previous finding from barrel neurons that, in general, initially smaller responses are affected proportionately more by the addition of BMI (Kyriazi *et al.*, 1996a). A second point is that pre-drug AW ON responses are much larger in infragranular regular-spike units than in regular-spike units of the middle and upper layers, whereas the PW responses do not differ significantly. This laminar difference in RF organization is particularly clear when the relative sizes of adjacent and principal whisker responses are examined. The left panel of Figure 3 plots RF focus, a normalized measure of AW response magnitude (see Methods), as a function of cortical depth. Note that RF focus broadens (increases in value) considerably as unit recordings are obtained at depths > 1000 μm.

Population data are summarized in the histograms in the right panel of Figure 3 (solid bars), and show that RF focus is narrowest in the barrel, broader in the supragranular layers, and broader still in the infragranular units. Similarly (data not shown), the RF focus of two studied infragranular fast-spike units is greater than those of middle layer fast-spike units ($n = 8$) (0.73 ± 0.01 vs 0.50 ± 0.08, $p = 0.026$ in group t-test). For the infragranular regular-spike units, the increase in RF focus under BMI is small, and not statistically significant. By contrast, supragranular and middle layer units display significant increases with the introduction of BMI (both p values ≤ 0.001). The two infragranular fast-spike units showed no increase in RF focus with BMI, a result that differed significantly from the increases seen in middle layer fast-spike units ($p < 0.05$; data not shown).

Each of the 26 middle layer regular-spike units showed a broadening of RF focus with BMI application. On the other hand, three of 20 supragranular regular-spike units and seven of 17 infragranular regular-spike units had *narrower* RF focuses in the presence of BMI. The coefficient of variation (SD/mean) for the change in RF focus was 0.58 for layer IV, 1.18 for supragranular units, and 3.53 for infragranular units. As these findings suggest, the infragranular regular-spike units were particularly heterogeneous. Six of these units had very high spontaneous activity ($\geq$ 6.5 times the median value), tended also to have the relatively largest AW responses (RF focus of 0.85), and their mean RF focus was unchanged with BMI (RF focus of 0.86). They were found at microdrive depth readings between 1022 and 1388 μm. Four of these six units showed the narrowing of RF focus under BMI, which may, however, be mostly a statistical phenomenon, since their AW responses were so large to begin with (i.e., with initial responses almost as large as those of the PW, their proportional increases with BMI are nearly the same as those of the PW, sometimes being more, and sometimes less). The 11 other infragranular regular-spike units, which had much smaller spontaneous activities, nevertheless still possessed relative AW response magnitudes significantly greater than those in other layers (RF focus of 0.52), which increased slightly, and insignificantly, with BMI addition (to 0.61; $p = 0.243$). Additional evidence for infragranular heterogeneity is the finding that the six highly spontaneously active regular-spike units showed a strong correlation between spontaneous activity and AW ON response ($R = 0.936$, $p = 0.003$), whereas the other 11 units tended to show an inverse correlation ($R = -0.495$, $p = 0.061$).

BMI's effects on surround inhibition

We examined inhibitory RF characteristics through the use of a condition-test paradigm (see Methods) wherein the AW was deflected 30 ms prior to the PW. The left panel of Figure 4 shows the changes

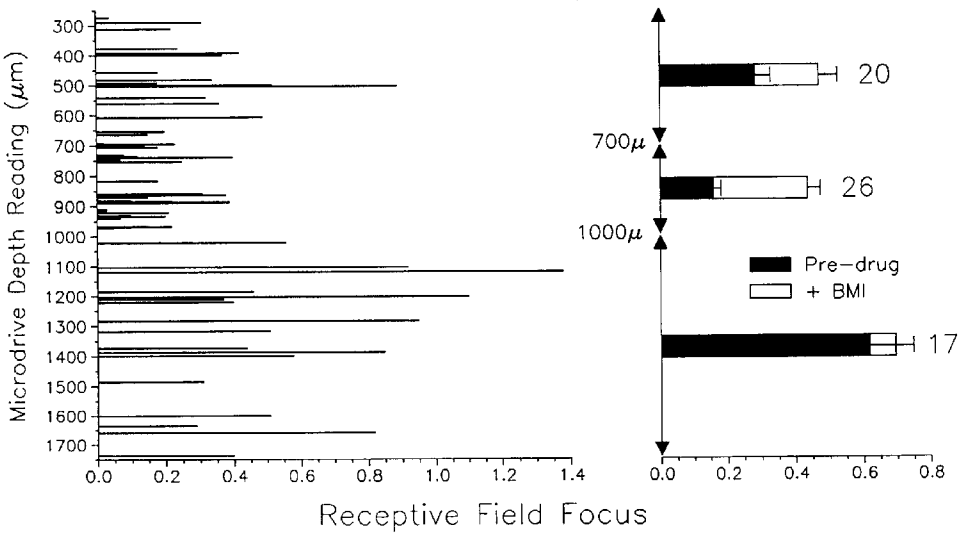

FIGURE 3. Receptive field (RF) focus as a function of cell depth, and the effect of bicuculline methiodide (BMI) addition. RF focus is the ratio of the caudally adjacent whisker ON response (averaged over all eight deflection angles) to that of the principal whisker. Note that the RF focus was always below 0.4 between microdrive readings of 650 and 1000 μm. In two cases, the RF focus was greater than 1.0, indicating that the adjacent whisker produced a greater response than the nominal principal whisker. These two units were from penetrations near the barrel edge, and so it is unclear whether the abnormally high RF focus values are due to the electrode having moved into the area between neighboring columns, or to statistical fluctuation. The right panel shows means and standard errors of the population data, grouped as indicated by the arrows.

that BMI caused in the CT ratios of individual regular-spike units as a function of recording depth. Positive values, indicating that BMI diminished surround inhibition, were observed for all but four units, three of which were in the supragranular layers, and one, with only a slightly negative value, was located infragranularly. Accordingly, the coefficient of variation for the change in the CT ratio was smallest in layer IV (0.47), largest in the supragranular units (1.45), and intermediate for the infragranular regular-spike units (0.82). The rightmost panel plots the average CT ratios of the three layers before and after BMI addition. CT ratios were similar in all three layers prior to BMI application (Kruskal–Wallis ANOVA; differences not significant), and BMI produced a statistically significant reduction of surround inhibition in each layer (pair *t*-tests: $p = 0.023, < 0.001$, and < 0.001, respectively, for upper, middle and lower layer regular-spike units). The loss of surround inhibition was greatest in the middle layer, although significantly so only with respect to the infragranular units. The 11 infragranular units with low spontaneous activity had much lower pre-drug CT ratios than the six highly active units (0.34 vs 0.59), but both subgroups showed similar magnitude, modest changes with BMI addition (CT ratios increased to 0.49 and 0.72, respectively). Considered separately, both subgroups were still significantly less affected by BMI than were barrel units (low spontaneous activity subgroup, Kruskal–Wallis ANOVA, $p = 0.009$ for the change in surround inhibition, $p = 0.028$ for the change in RF focus; high spontaneous activity subgroup, $p = 0.046$ and $p = 0.002$, respectively).

Discussion

This study compared the effects of BMI on neurons at different depths in whisker-related cortical columns. The comparability of effects among different units is based on the standardization procedures we used to adjust ejection currents independently for each unit. We interpret BMI's effects in terms of direct action on the neuron under study, rather than to secondary effects resulting from any BMI action on neighboring neurons (but see below). We base this on the low BMI currents used (0–20 nA) and to a presumed high degree of convergence onto cortical neurons, which may suggest a negligible influence of the inputs of only one or a few neighboring neurons. Also, we have chosen to classify neurons into three laminar groups, ignoring possible subgroupings, e.g., layer V vs VI, that are likely to be important with respect to the underlying circuitry, but might only be revealed in studies that are more extensive or that utilize different RF assessment protocols. In terms of the present data, the findings with respect to laminar location are robust, and, as can be appreciated by examination of the left-hand panels of Figures 3 and 4, conclusions are not sensitive to potential misclassification of units recorded near laminar boundaries.

Excitatory RFs and serial processing

As shown in previous studies of whisker/barrel cortex, the anatomically defined PW (i.e., the somatotopically matching whisker, determined histologically) evokes the strongest excitatory response and also the shortest latency response (Simons, 1978; Armstrong-James *et al.*, 1992).

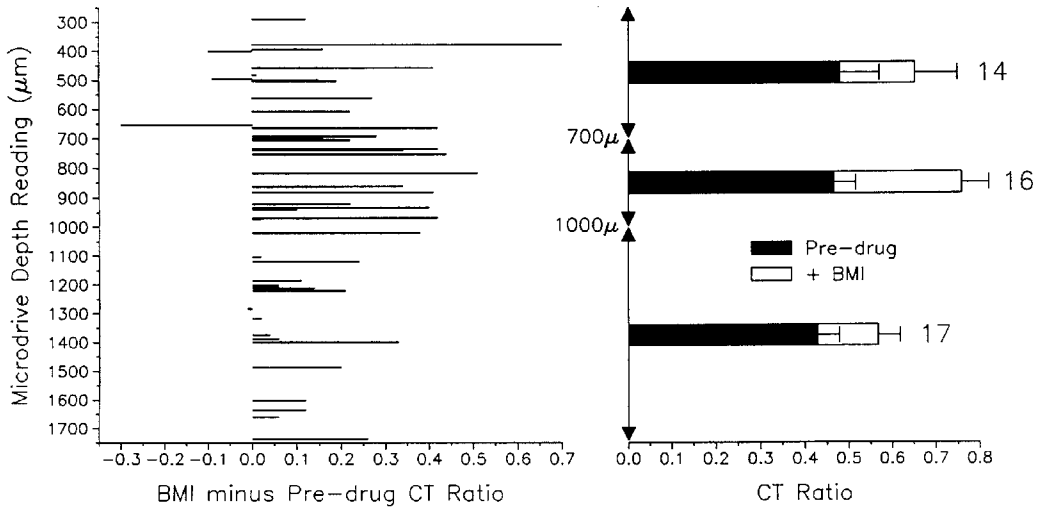

FIGURE 4. Surround inhibition as a function of cell depth, and the effect of bicuculline methiodide (BMI) addition. The left panel shows the change in condition-test ratios (CT ratios) caused by BMI addition, as a function of depth. A positive value on the *x*-axis indicates a lessening of surround inhibition. The right panel shows the means and standard errors of CT ratios of the three layers. Pre-drug and BMI results are superimposed; open portions of the bars constitute the differences. The change seen in the granular layer is significantly greater than that in the infragranular layer ($p = 0.002$); the difference between it and the supragranular layer is not significant ($p = 0.129$).

Although BMI application caused the greatest proportional increases in the initially smallest responses, the absolute magnitudes of PW responses remained larger than those of the AW, and latencies were unaffected, as also found in cat SmI cortex (Alloway *et al.*, 1989). The finding that BMI did not alter response latencies indicates that it does not increase activity by 'unmasking' a different set of inputs having different time courses.

That supragranular SmI units tend to respond at longer latencies than granular and infragranular units was demonstrated previously in intracellular (Carvell and Simons, 1988; Agmon and Connors, 1992) and extracellular (Armstrong-James *et al.*, 1992; Alloway *et al.*, 1993) studies, and this was also found in the present extracellular recordings. The supragranular ON response PSTHs differed also in having a more flattened appearance than those of the other layers (see Figure 1), which may be due to a combination of two factors. Firstly, supragranular units receive a larger proportion of their excitatory drive in a more temporally dispersed fashion, from intracolumnar layer IV inputs (e.g., see Johnson and Alloway, (1996)) and intercolumnar, horizontal inputs from other layer II/III units. Secondly, they may have slower membrane kinetics generally (e.g., in cat motor cortex, layer II/III cells have slower *EPSPs* and *IPSPs* than layer V units; van Brederode and Spain (1995)); this latter property would also contribute to their longer latencies.

No significant timing differences were found between barrel regular-spike units and infragranular regular-spike units, similar to the findings obtained by Armstrong-James *et al.* (1992), using urethan-anesthetized rats, of a roughly equal latency to PW deflection of layer IV and layer Vb neurons. Armstrong-James *et al.* also found the PW responses of supragranular units to be delayed 2–3 ms relative to the other layers, similar to the results reported here. An unresolved question is whether the longer AW latencies are due to delayed input, weak input, or both (weak input requiring more temporal summation to achieve spike threshold).

The relative size of the AW vs PW response, which we quantified as RF 'focus', clearly varied as a function of cortical depth. AW responses were relatively smallest in layer IV, largest in the infragranular layers and of intermediate value superficially. This is consistent with findings from previous studies of rodent somatosensory cortex where RF size *per se* was measured; RFs were smallest in layer IV and largest in layers V/VI (Simons, 1978; Chapin, 1986). It is generally thought that the comparatively large AW responses in non-granular layers reflect intercolumnar connections.

BMI changed RF focus by disproportionately enhancing the AW responses that were initially smallest. In the barrel, AW regular-spike unit responses, which were on average ~0.2 spikes/stimulus, increased six-fold in the presence of BMI; infragranular AW responses, initially at ~0.8 spikes/stimulus, showed only a two-fold increase. Because PW responses increased equivalently across all depths, the net effect of BMI was to blur the laminar differences in RF focus. We do not view the greater relative BMI-induced increase in barrel AW responses in terms of those units being under more profound inhibition than infragranular units, but rather as a result of the non-linearity of the spike thresholding mechanism (see Kyriazi *et al.* (1996a)). Thus, the same amount of BMI-induced membrane depolarization would be expected to have a relatively greater effect on the barely threshold barrel AW responses than on the more strongly

suprathreshold infragranular AW responses. Presumably, very weak infragranular regular-spike unit responses, such as to deflection of more distant whiskers, would show almost the same six-fold proportional increases with BMI as do the barrel regular-spike unit AW responses.

Our finding that BMI-induced changes in RF focus were greatest in layer IV may seem to conflict with the finding of Dykes *et al.* (1984) that BMI-induced RF expansion in cat somatosensory cortex was least at these depths. RF focus and RF size, however, measure different aspects of the excitatory RF. The former is a normalized measure of AW response magnitude, whereas RF size is a non-relational measure of the absolute spatial extent of the RF. These two measures can, in principle, change independently. For example, the relative strength of an AW's response can increase without an increase in the total number of whiskers that excite the neuron. In the barrels, where the maximum RF size may be limited by thalamocortical input (see Simons and Carvell (1989); Land *et al.* (1995)), BMI-induced RF expansion may be less than in non-granular layers where extensive intercolumnar connections (Bernardo *et al.*, 1990; Hoeflinger *et al.*, 1995) are a potential source of normally subthreshold excitatory inputs from distant whiskers.

Surround inhibition and serial processing

Interestingly, fast-spike units in the barrels display the shortest latency PW and AW responses, whose earliest onset times are almost indistinguishable (see Figure 1). This is consistent with the finding of Agmon and Connors (1992), in a thalamocortical slice preparation, that all fast-spike units receive short latency, monosynaptic input from the thalamus, and our previous finding that thalamic neurons tend to respond to both PW and AW stimulation, although less vigorously to the latter (Simons and Carvell, 1989). According to our model (Kyriazi and Simons, 1993), the fast-spike unit response to AW deflection, although weaker than its PW response, is nevertheless rapid enough and substantial enough to produce the condition-test inhibition measured in the present study, and it is the effectiveness of this fast-spike unit output that is diminished by BMI, especially with regard to barrel neurons.

BMI caused a reduction in AW-evoked, surround inhibition in all but four of 47 regular-spike units tested. Although pre-drug CT ratios were equivalent among layers, the magnitude of BMI's effect varied with laminar location. Disinhibition, measured as an increase in CT ratio, was greatest for barrel neurons, least for infragranular neurons, and intermediate in the case of supragranular neurons. A similar ordering of laminar-dependent differences has been observed in other measures of RF organization in the rat somatosensory cortex (Lamour *et al.*, 1983; Ito, 1985; Alloway *et al.*, 1993; and see also below). Such findings have generally been thought to reflect a sequential flow of information within the cortical column, beginning at middle depths (for reviews, see Armstrong-James (1995) and Simons (1995)), where thalamocortical afferents terminate most densely (see White (1989)). Barrel neurons receive most of their extrinsic inputs from thalamic 'barreloid' neurons, which as a population display relatively weak surround inhibition (Simons and Carvell, 1989). As discussed above, local circuitry in layer IV, involving robust fast-spike unit responses to AW stimulation, is thought to be sufficient to create, *de novo*, strong surround inhibition in barrel neurons (Simons and Carvell, 1989; Kyriazi and Simons, 1993). These views are consistent with the present findings that local application of BMI greatly reduces surround inhibition in these neurons (and see below).

Supra- and infragranular neurons receive much of their excitatory input from neurons that have already acquired surround inhibition, i.e., from layer IV barrel cells and from other layer II/III and layer V/VI neurons (for review, see White (1989)). Thus, the surround inhibition displayed by many supra- and infragranular units is largely built into their input, and, therefore, relatively immune to BMI applied locally near their somata. Sato *et al.* (1995, 1996) offered a similar explanation for their findings in monkey primary visual cortex that direction and orientation selectivity of layer IV neurons were less affected by BMI than were layer IV units: the direction and orientation properties of the layer VI cells were thought to reflect the RF characteristics of their excitatory inputs from other cortical layers. However, our findings with BMI regarding RF focus changes suggest caution in drawing conclusions about serial processing based on relational excitatory RF quantities, such as in the studies of Sato *et al.*. If units in one layer are much more responsive than those elsewhere, then BMI's lesser proportional effects on initially larger responses would inevitably result in less of a BMI effect in that layer.

Another possible explanation for the lesser effect of BMI on infragranular units lies in the great distance of their apical dendrites from their somata. Alloway *et al.* (1989) and Alloway and Burton (1991) speculated from BMI experiments in cat and monkey primary somatosensory cortex that there may be a spatial segregation of inputs onto pyramidal cells, such that long-range horizontal connections may tend to be made onto more distal regions of the dendrites. If the inhibitory inputs, also driven by long-range horizontal connections, and mediating some degree of surround inhibition, are made on the distant, apical dendrites, then that degree of surround inhibition would be relatively unaffected by small amounts of BMI applied close to the soma.

A trivial explanation for the lesser effect of BMI on surround inhibition in the infragranular units

might be that their conditioned-test responses are initially larger than those in the barrel and, as a result, are proportionally less affected by it, just as their RF focus values change less than those in the barrel, where the AW responses are initially much smaller. The infragranular regular-spike units' conditioned-test responses were, however, only slightly, and insignificantly, greater than those in the barrel (0.67 vs 0.62 spikes/stimulus). Moreover, the low and high spontaneous activity infragranular units showed almost the same magnitude change in surround inhibition with BMI (CT ratio increases of 0.14 vs 0.13), despite having very different conditioned-test response magnitudes (0.48 vs 1.02 spikes/stimulus; Kruskal–Wallis ANOVA, $p = 0.035$) and initial CT ratios (0.34 vs 0.59, Kruskal–Wallis $p = 0.016$). Even if these infragranular regular-spike unit subgroups were to consist of pyramidal vs non-pyramidal neurons (see below), their responses to BMI would still be significantly different than those of barrel neurons. Thus, the lesser effect of BMI in infragranular units is not a ceiling phenomenon, and we conclude that it constitutes evidence for serial processing, i.e., that barrel regular-spike units perform an initial transformation of thalamocortical input, which is then passed on to the upper and lower layer units.

What are local excitatory connections in the barrel doing?

Given that ~85% of the excitatory synapses onto barrel regular-spike units are thought to arise from same-barrel regular-spike units (see White (1989)), one might expect barrel regular-spike units to be more immune to BMI-induced loss of surround inhibition than they in fact are. In other words, one might expect barrel regular-spike units to behave more like non-granular layer regular-spike units if they truly receive the bulk of their inputs from other barrel neurons, which themselves must provide a high level of 'built-in' surround inhibition. But unpublished computer simulations with a model that has produced realistic output in a variety of settings (see Kyriazi and Simons (1993), Kyriazi *et al.* (1996a)) indicate that barrel regular-spike units deprived of such recurrent excitation (but retaining inhibitory input from barrel fast-spike units) cannot be made to function properly. Specifically, they display less than the normal amount of surround inhibition (CT ratio of 0.66 vs 0.48), and when inhibitory inputs to individual modeled neurons are weakened sufficiently to produce a doubling of PW ON responses, thus simulating BMI addition, the lessening of surround inhibition is excessive (CT ratio of 0.94 vs 0.80). This suggests that feedback excitatory inputs of barrel regular-spike units onto one another do contribute substantially to their surround inhibition.

The above discussion assumes that the iontophoresed BMI diffuses equivalently in all layers, and does not disinhibit a greater proportion of neighboring excitatory cells in the layer IV barrel. There were no significant differences in the BMI currents used in the various layers, and the degree to which neighboring cells were affected was minimized by the small BMI ejection currents used. However, because of the small size and high packing density of the barrel stellate cells, the same spread of BMI there would be expected to affect more neurons than it would in non-granular layers. If the diffusion of BMI were such as to affect a significant portion of the barrel neuron network, many of the excitatory inputs to the studied cell would also have lost some of their *de novo* surround inhibition, contributing to the greater disinhibitory effect of BMI on barrel regular-spike units. We showed previously, however, using modeled barrel neurons, that such secondary effects need not be invoked to accurately reproduce BMI's effects on a variety of excitatory and inhibitory RF characteristics (Kyriazi and Simons, 1993; Kyriazi *et al.*, 1996a). In addition, in the present study there was no correlation whatsoever, in any or all of the layers, between BMI current and the extent of loss of surround inhibition, suggesting that if these doses of BMI do affect neighboring neurons, any secondary effects on the studied neuron are eliminated by other factors. Nevertheless, the possibility cannot be excluded that some of the greater effect that BMI has on barrel neurons may result from its affecting more neighboring neurons there than it does in non-granular layers.

Intralaminar heterogeneity

An examination of the left-hand panel of Figure 4 shows that BMI's effects on surround inhibition are more heterogeneous within the supragranular and infragranular populations than among barrel neurons. Several fast-spike unit-like properties of the highly spontaneously active subset of infragranular regular-spike units (i.e., their large RF focus values, high CT ratios, and great responsiveness in general) suggest the possibility that they may, in fact, not be pyramidal neurons, but inhibitory interneurons. That possibility aside, we interpret the greater non-granular layer heterogeneity with respect to BMI's effects to mean that individual pyramidal neurons differ greatly in terms of the extent to which their surround inhibition is created *de novo* from direct inhibitory inputs rather than being embedded in their excitatory inputs, as discussed above. Figure 5 is a highly schematic diagram that shows two circuits in the supragranular layer whose respective pyramidal neurons—P1 and P2—would differ with regard to BMI's ability to lessen surround inhibition. These two pyramidal neurons lie within the same column and receive different proportions of intra- and intercolumnar excitatory drive. P1 re-

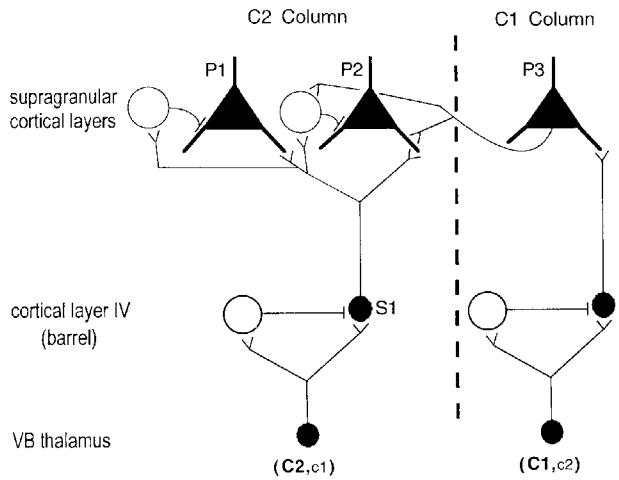

FIGURE 5. Highly schematic circuit diagram to illustrate one possible explanation of the heterogeneity of bicuculline methiodide's (BMI) effect on adjacent whisker evoked surround inhibition in supragranular neurons. The C1 and C2 whisker's cortical columns are shown, along with their ventrobasal (VB) thalamic barreloid inputs, and are separated by the vertical dashed line. The parenthetical expressions below the VB neurons are meant to indicate the relative strengths of their responses to principal and adjacent whisker stimuli. Unfilled neurons are inhibitory, filled are excitatory. S1, spiny stellate cell in the C2 barrel; P1, P2 and P3 are supragranular pyramidal cells. P1's surround inhibition is almost entirely embedded in its excitatory input from S1. That of P2 may be stronger, as some of it is generated *de novo* via P3's drive onto P2's inhibitory neuron, and would be expected to be more susceptible to BMI.

ceives only intracolumnar drive, represented in the diagram by the input from spiny stellate cell S1 in the parent barrel, while P2 and its inhibitory neighbor receive additional drive from neuron P3 in the adjacent column, which receives its excitatory input from a spiny stellate cell in its own column's barrel.

Both spiny stellate neurons display strong surround inhibition as a result of direct inhibitory inputs from interneurons within their respective barrels, and both show substantial loss of surround inhibition with local application of BMI. The inhibitory inputs from those interneurons are driven by the multiwhisker inputs coming from their corresponding ventrobasal thalamic barreloids. In contrast to barrel neuron S1, neuron P1 has little surround inhibition except that which is built into its excitatory inputs coming from S1. Although P1 does receive direct inhibitory inputs, and thus is affected by BMI, these inputs are not strongly driven by deflection of the C1 AW, and thus the *surround* inhibition displayed by P1 would be largely insensitive to BMI. The surround inhibition of P2, on the other hand, would be much more susceptible to BMI application.

The circuitry for neurons P1 and P2 shown in Figure 5 suggests that one might expect to find a correlation between a supragranular neuron's AW response magnitude and the extent of surround inhibition that that AW may elicit; interestingly, a hint of a such a correlation exists ($R = 0.31$, $p = 0.14$, $n = 14$), and if one looks at the normalized AW response (i.e., RF focus), a weak correlation is found ($R = 0.47$, $p = 0.044$, $n = 14$). One might also expect to find a correlation between AW response magnitude and the extent to which BMI affects surround inhibition. None is present, however, perhaps for the following reason. If pyramidal neuron P2 were to not receive direct excitatory inputs from P3, but if its inhibitory input did so from the type of connection shown, or if its inhibitory input came directly from the C1 column by way of lateral spread of inhibitory cell axons (see van Brederode and Spain (1995), Salin and Prince (1996)), then in either case it would tend to diminish any correlation between the magnitude of the C1 whisker's excitatory and inhibitory effects, resulting, perhaps, in the weak to insignificant p values reported above.

Other differences in circuitry are known to exist which would lead to pyramidal cell response heterogeneity. For example, infragranular pyramidal neurons are known to differ in terms of the relative amounts of thalamocortical vs other excitatory synaptic inputs (White, 1989). Thalamic inputs contain the least amount of embedded surround inhibition. Negation of surround inhibition by BMI would, therefore, be greater in pyramidal cells participating in mini-circuits that receive more thalamocortical drive, because the drug would be antagonizing the GABA receptors that generate much of the surround inhibition in that neuron. Corticothalamic neurons, which receive relatively large proportions of thalamocortical synapses, might exhibit greater BMI-induced loss of surround inhibition than ipsilateral corticocortical or corticostriatal neurons, which receive comparatively less, direct thalamic drive.

Acknowledgments

This work was supported by National Institutes of Neurological Diseases and Stroke Grant NS-19950.

References

AGMON, A., and B.W. CONNORS (1992) Correlation between intrinsic firing patterns and thalamocortical synaptic responses of neurons in mouse barrel cortex. *J Neurosci* **12**: 319–329.

AKHTAR, N.D., and P.W. LAND (1991) Activity-dependent regulation of glutamic acid decarboxylase in the rat barrel cortex: effects of neonatal versus adult sensory deprivation. *J Comp Neurol* **307**: 200–213.

ALLOWAY, K.D., and H. BURTON (1991) Differential effects of GABA and bicuculline on rapidly- and slowly-adapting neurons in primary somatosensory cortex of primates. *Exp Brain Res* **85**: 598–610.

ALLOWAY, K.D., M.J. JOHNSON, and M.B. WALLACE (1993) Thalamocortical interactions in the somatosensory system: interpretations of latency and cross-correlation analyses. *J Neurophysiol* **70**: 892–908.

ALLOWAY, K.D., P. ROSENTHAL, and H. BURTON (1989) Quantitative measurements of receptive field changes during antagonism of GABAergic transmission in primary somatosensory cortex of cats. *Exp Brain Res* **78**: 514–532.

ARMSTRONG-JAMES, M. (1995) The nature and plasticity of sensory processing within adult rat barrel cortex. In *Cerebral Cortex*, Vol. 11, *The Barrel Cortex of Rodents*, E.G. JONES, and I.T. DIAMOND, eds. pp. 333–373, Plenum Press, New York.

ARMSTRONG-JAMES, M.K. FOX, and A. DAS-GUPTA (1992) Flow of excitation within rat barrel cortex on striking a single vibrissa. *J Neurophysiol* **68**: 1348–1358.

BERNARDO, K.L., J.S. MCCASLAND, T.A. WOOLSEY, and R.N. STROMINGER (1990) Local intra- and interlaminar connections in mouse barrel cortex. *J Comp Neurol* **291**: 231–255.

BRUMBERG, J.C. (1997) Local circuits and columnar organization in the somatosensory cortex. *Ph.D. Dissertation*, University of Pittsburgh.

CARVELL, G.E., and D.J. SIMONS (1988) Membrane potential changes in rat SmI cortical neurons evoked by controlled stimulation of mystacial vibrissae. *Brain Res* **448**: 186–191.

CHAPIN, J.K. (1986) Laminar differences in sizes, shapes, and response profiles of cutaneous receptive fields in the rat SI cortex. *Exp Brain Res* **62**: 549–559.

DYKES, R.W., and Y. LAMOUR (1988) An electrophysiological study of single somatosensory neurons in rat granular cortex serving the limbs: a laminar analysis. *J Neurophysiol* **60**: 703–724.

DYKES, R.W., P. LANDRY, R. METHERATE, and T.P. HICKS (1984) Functional role of GABA in cat primary somatosensory cortex: shaping receptive fields of cortical neurons. *J Neurophysiol* **52**: 1066–1093.

HOEFLINGER, B.F., C.A. BENNETT-CLARKE, N.L. CHIAIA, H.P. KILLACKEY, and R.W. RHOADES (1995) Patterning of local intracortical projections within the vibrissae representation of rat primary somatosensory cortex. *J Comp Neurol* **354**: 551–563.

ITO, M. (1985) Processing of vibrissa sensory information within the rat neocortex. *J Neurophysiol* **54**: 479–490.

JOHNSON, M.J., and K.D. ALLOWAY (1996) Cross-correlation analysis reveals laminar differences in thalamocortical interactions in the somatosensory system. *J Neurophysiol* **75**: 1444–1457.

KYRIAZI, H.T., G.E. CARVELL, J.C. BRUMBERG, and D.J. SIMONS (1996a) Quantitative effects of GABA and bicuculline methiodide on receptive field properties of neurons in real and simulated whisker barrels. *J Neurophysiol* **75**: 547–560.

KYRIAZI, H.T., G.E. CARVELL, J.C. BRUMBERG, and D.J. SIMONS (1996b) Effects of baclofen and phaclofen on receptive field properties of rat whisker barrel neurons. *Brain Res* **712**: 325–328.

KYRIAZI, H.T., G.E. CARVELL, and D.J. SIMONS (1994) OFF response transformations in the whisker/barrel system. *J Neurophysiol* **72**: 392–401.

KYRIAZI, H.T., and D.J. SIMONS (1993) Thalamocortical response transformations in simulated whisker barrels. *J Neurosci* **13**: 1601–1615.

LAMOUR, Y., P. DUTAR, A. JOBERT, and R.W. DYKES (1988) An iontophoretic study of single somatosensory neurons in rat granular cortex serving the limbs: a laminar analysis of glutamate and acetylcholine effects on receptive-field properties. *J Neurophysiol* **60**: 725–750.

LAMOUR, Y., G. GUILBAUD, and J.C. WILLER (1983) Rat somatosensory (SmI) cortex: II. Laminar and columnar organization of noxious and non-noxious inputs. *Exp Brain Res* **49**: 46–54.

LAND, P.W., S.A. BUFFER, JR., and J.D. YASKOSKY (1995) Barreloids in adult rat thalamus: three dimensional architecture and relationship to somatosensory cortical barrels. *J Comp Neurol* **355**: 573–588.

SALIN, P.A., and D.A. PRINCE (1996) Electrophysiological mapping of $GABA_A$ receptor-mediated inhibition in adult rat somatosensory cortex. *J Neurophysiol* **75**: 1589–1600.

SATO, H., N. KATSUYAMA, H. TAMURA, Y. HATA, and T. TSUMOTO (1995) Mechanisms underlying direction selectivity of neurons in the primary visual cortex of the macaque. *J Neurophysiol* **74**: 1382–1394.

SATO, H., N. KATSUYAMA, H. TAMURA, Y. HATA, and T. TSUMOTO (1996) Mechanisms underlying orientation selectivity of neurons in the primary visual cortex of the macaque. *J Physiol* **494**(3): 757–771.

SIMONS, D.J. (1978) Response properties of vibrissa units in the rat SI somatosensory neocortex. *J Neurophysiol* **41**: 798–820.

SIMONS, D.J. (1983) Multi-whisker stimulation and its effects on vibrissa units in rat SmI barrel cortex. *Brain Res* **276**: 178–182.

SIMONS, D.J. (1985) Temporal and spatial integration in the rat SI vibrissa cortex. *J Neurophysiol* **54**: 615–635.

SIMONS, D.J. (1995) Neuronal integration in the somatosensory whisker/barrel cortex. In *Cerebral Cortex*, Vol. 11, E.G. JONES, and I.T. DIAMOND, eds., pp. 263–297, Plenum Press, New York.

SIMONS, D.J., and G.E. CARVELL (1989) Thalamocortical response transformation in the rat vibrissa/barrel system. *J Neurophysiol* **61**: 311–330.

SIMONS, D.J., G.E. CARVELL, A.E. HERSHEY, and D.P. BRYANT (1992) Responses of barrel cortex neurons in awake rats and effects of urethane anesthesia. *Exp Brain Res* **91**: 259–272.

SIMONS, D.J., and P.W. LAND (1987) A reliable technique for marking the location of extracellular recording sites using glass micropipettes. *Neurosci Letts* **81**: 100–104.

SIMONS, D.J., and T.A. WOOLSEY (1979) Functional organization in mouse barrel cortex. *Brain Res* **165**: 327–332.

VAN BREDERODE, J.F.M., and W.J. SPAIN (1995) Differences in inhibitory synaptic input between layer II–III and layer V neurons of the cat neocortex. *J Neurophys* **74**: 1149–1166.

WELKER, C. (1976) Receptive fields of barrels in the somatosensory neocortex of the rat. *J Comp Neurol* **166**: 173–189.

WHITE, E.L. (1989) *Cortical Circuits: Synaptic Organization of the Cerebral Cortex*, Birkhauser, Boston.

WOOLSEY, T.A., and H. VAN DER LOOS (1970) The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. *Brain Res* **17**: 205–242.