Plasticity of Excitation and Inhibition in the Receptive Field of Primary Auditory Cortical Neurons after Limited Receptor Organ Damage

Permanent receptor organ damage can cause plasticity of topographic cortical maps of that receptor surface while temporary receptor organ damage, and conditions mimicking such damage, can unmask new excitatory inputs in central sensory neurons receiving input from that receptor surface. Cortical plasticity is associated with an anatomically or pharmacologically defined decrease in inhibition in cortex. It is therefore widely proposed that a reduction in central inhibition underlies cortical neural plasticity. Here I demonstrate that small receptor organ damage results, in primary auditory cortical (A1) neurons, in loss of one component of functionally defined afferent inhibition but unmasking of another component of afferent inhibition along with new excitatory responses. Overall, there did not appear to be any change in the strength of afferent inhibition or in the strength of excitation. Thus, auditory receptor organ damage can unmask new excitatory inputs as well as inhibitory inputs from within the receptive field of the neurons.

Introduction

Damage to sensory receptor surfaces can cause dramatic changes in excitatory responses of cortical neurons serving that sensory modality. One such change is the plasticity in topographic receptor-surface maps in adult auditory, visual or somatosensory cortex, that occurs after permanent damage to restricted parts of the appropriate receptor surface (Rasmusson, 1982; Merzenich et al., 1983, 1984; Kelahan and Doetsch, 1984; Calford and Tweedale, 1988; Robertson and Irvine, 1989; Kaas et al., 1990; Heinen and Skavenski, 1991; Pons et al., 1991; Chino et al., 1992; Gilbert and Weisel, 1992; Rajan et al., 1993; Schwaber et al., 1993; Schmid et al., 1996). Similar plasticity is seen of the motor cortex map of the musculature after permanent damage to restricted motoneurons (Sanes et al., 1990). A related change in sensory cortical neurons is the rapid unmasking of previously unexpressed excitatory inputs that occurs after acute peripheral damage (Kelahan and Doetsch, 1984; Calford and Tweedale, 1988, 1991; Faggin et al., 1997) or after conditions mimicking damage (Pettet and Gilbert, 1992).

Anatomically defined loss of inhibition is associated with sensory cortex plasticity (Hendry and Jones, 1986; Hendry and Kennedy, 1986; Ribak and Robertson, 1986; Warren et al., 1989; Welker et al., 1989; Hendry et al., 1990; Akhtar and Land, 1991; Garraghty et al., 1991; Rausell et al., 1992; Skangiel-Kramska et al., 1994; Rosier et al., 1995; Micheva and Beaulieu, 1995; D’Amelio et al., 1996; Kyriazi et al., 1996) and plasticity of the motor cortex map is achieved by pharmacological blockade of intracortical inhibition (Jacobs and Donoghue, 1991). Hence, many models (Ribak and Robertson, 1986; Welker et al., 1989; Hendry et al., 1990; Jacobs and Donoghue, 1991; Garraghty et al., 1991; Rausell et al., 1992; Jones, 1993; Rosier et al., 1995; Ralston et al., 1996) of cortical map plasticity and of the unmasking of new excitatory responses propose that loss of inhibition plays a critical permissive role. Neural network models (Pearson et al., 1987; Jenison, 1997) of cortical map plasticity explicitly include a decrease in inhibitory strength as a critical part of the models. However, there is evidence to suggest that damage to receptor surfaces does not cause a simple decrease in inhibitory central sensory neurons. Studies in raccoons reported that the immediate effect (within 1 week) of digit amputation was unmasking of inhibition from adjacent digits in cortical (Rasmusson and Turnbull, 1983; Turnbull and Rasmusson, 1990; Rasmusson et al., 1992) and thalamic (Rasmusson et al., 1993) neurons formerly receiving input from the amputated digit. Such unmasking of ‘off-focus inhibition’ was found after either permanent (Rasmusson and Turnbull, 1983; Turnbull and Rasmusson, 1990; Rasmusson et al., 1992) or temporary denervation (Rasmusson et al., 1993). Since temporary and permanent receptor organ damage (Kelahan and Doetsch, 1984; Calford and Tweedale, 1988, 1991; Faggin et al., 1997) or conditions mimicking such damage (Pettet and Gilbert, 1992) can cause immediate unmasking of ‘new’ excitatory inputs in many (if not all) central neurons, an immediate unmasking of inhibitory inputs is inconsistent with the hypothesis that a simple reduction in inhibition directly underlies unmasking of new excitatory responses in sensory cortex. Similarly, short-term and relatively moderate damage to the auditory receptor organ has been shown to produce immediate changes in primary auditory cortical (A1) neurons that could not always be explained as due to a reduction in inhibition (Calford et al., 1993). In some A1 neurons there appeared to be an increase in inhibition relative to excitation, while in other neurons there appeared to be differential effects on excitation and inhibition in different parts of the neuron’s receptive field (Calford et al., 1993). Again, these results do not suggest a simple reduction in inhibition consequent on receptor organ damage.

In visual, auditory and somatosensory systems stimulus-driven (afferent) inhibition to central neurons is functionally defined as consisting of two components (Laskin and Spencer, 1979). In-field inhibition arises from within the same receptor area that also provides excitation to the neuron. Additionally, in many neurons surround inhibition is defined as present when inhibition extends beyond the excitatory RF. The studies of peripheral damage-induced changes in inhibition in central sensory neurons do not distinguish between these forms of inhibition, which appear to play different roles in shaping excitatory responses in central auditory and somatosensory neurons at least (Janig et al., 1977, 1979; Suga and Manabe, 1982; Martin and Dickson, 1983; Dykes et al., 1984; Suga and Tsuzuki, 1985; Alloway and Burton, 1986; Müller and Scheich, 1987, 1988; Rama et al., 1988; Alloway et al., 1989; Kaneko and Hiks, 1990; Oka and Hicks, 1990; Alloway and Burton, 1991; Caspary et al., 1991; Yang et al., 1992; Vater et al., 1992; Suga, 1995; Calford and Semple, 1995; Palombi and Caspary, 1996; Palombi...
et al., 1996; Brosch and Schreiner, 1997). I have previously demonstrated (Rajan, 1998) that small auditory receptor organ damage causes loss of surround inhibition in single cortical cells. The test cells with loss of surround inhibition allowed examination of whether receptor organ damage caused a uniform decrease in inhibition (as reflected in all forms of afferent inhibition) in unmasking excitatory responses.

Materials and Methods

Animal Treatment and Measurement of Hearing Sensitivity

Procedures involved in animal treatment and cortical recordings have been detailed elsewhere (Rajan, 1998). In brief, 6 test and 21 control adult cats weighing 2.5–4 kg were tested under procedures approved by the Monash University Standing Committee on Ethics in Animal Experimentation and conforming to the guidelines of National Health and Medical Research Council of Australia. Test cats had small, but statistically significant, unilateral chronic cochlear hearing losses (either noise-induced or idiopathic, produced some months prior to these experiments; equal numbers of the two types being used) over some well-defined frequency range between 5 and 24 kHz, and were tested concurrent with control animals with bilaterally normal cochlear hearing sensitivity. Animals were anaesthetized (60 mg/kg) and maintained with Nembutal (2–3 mg/kg/h). Body temperature was maintained at 37.5 ± 0.5°C. Surgery (Rajan et al., 1993; Rajan, 1998) was carried out to measure cochlear hearing sensitivity bilaterally and to record from primary auditory cortex (A1). Cochlear hearing sensitivity was assessed by measuring thresholds for the compound action potential (CAP) of the auditory nerve at frequencies from 0.5 to 40 kHz (Rajan et al., 1991) and compared to normative laboratory data (Rajan et al., 1991).

Cortical Recordings

Initially the tonotopic organization of A1 (in test animals, A1 contralateral to the cochlea with hearing loss; in control animals, A1 in the left or right hemisphere) was quickly mapped across a wide rostrocaudal area with recordings from multi-unit clusters in the middle cortical layers, using low-impedance microelectrodes (Rajan et al., 1993; Rajan 1998). For each cluster, characteristic frequency (CF; frequency of greatest sensitivity) and CF-threshold were determined audiologically. These were confirmed by recording the excitatory response area (ERA; the frequency–intensity space eliciting excitatory responses) under computer control, using single tones varying in frequency and intensity over a large frequency–intensity matrix, with 5–20 repetitions of the matrix (Rajan et al., 1993; Rajan, 1998). Extracellular recordings (Rajan et al., 1993; Rajan, 1998) were then obtained from single, well-isolated A1 neurons, generally from the middle layers (III/IV), using tungsten-in-glass microelectrodes (impedances = 5–7 MΩ at 1 kHz). Neurons were from A1 regions approximately in the middle of the dorsoventral extent of A1 (Volkov and Galazuk, 1991; Sutter and Schreiner, 1995), as determined from mapping penetrations. Recording site locations with respect to cortical vasculature were marked on a high-resolution cortical photograph. Laminar location of cells was estimated by noting recording depth relative to the cortical surface, from the calibrated microdrive. Occasionally, electrolytic lesions were made and cortex recovered post-mortem for histology and identification of lesion sites relative to laminar boundaries.

Defining the Excitatory Response Area and the inhibitory areas in the RF

In each cell, first the ERA was defined under computer control (Rajan et al., 1993; Rajan, 1997, 1998), with 5–20 repetitions of a large frequency–intensity matrix of single tones, as detailed above. From the ERA the cell’s CF and threshold at CF were identified for defining inhibitory areas. Inhibitory areas were defined, under computer control, using a masking paradigm that involved presenting two successive tones. The second (probe) tone was fixed in frequency and intensity at levels excitatory to the cell (always at the cell’s CF, at a level 15–25 dB > CF threshold) whereas the first (masking) tone was varied in frequency and intensity over a large matrix, with 5–20 repetitions of the matrix in the two-tone combination. Probe and masking tones were generated by separate channels of a digital synthesis system and mixed electronically before being fed to the speaker in the ear canal contralateral to A1. To minimise subcortical contributions (R. Rajan, unpublished results) data here are for a delay of 50 ms between masker end and probe tone start.

Inhibitory areas were defined as the tonal frequency–intensity space causing a significant reduction (at α = 0.05) in probe responses in the two-tone condition compared to the probe-alone condition.

The suppression frequency–intensity area was overlaid on to the ERA, and surround inhibition (Laskin and Spencer, 1979) was defined to be present when the suppression area extended beyond the ERA boundaries by at least two test frequencies and over an intensity range of at least 20 dB (e.g. Figs 2a, 3a,b). If it did not extend beyond the ERA by these criteria but was confined within the ERA, the cell was defined as having only in-field inhibition from within the ERA (Laskin and Spencer, 1979) (e.g. Figs 2b, 3c–f). In all cases where surround inhibition was present, it was continuous with in-field inhibition (Figs 2a, 3a,b). If suppression was found only at one frequency within the ERA and did not extend over at least 20 dB, the cell was defined as not possessing any inhibition.

Bandwidths of Excitatory and Inhibitory Response Areas

Bandwidths of ERAs and of inhibitory areas (the total area of suppression of probe responses) were measured using the Q metric (Sutter and Schreiner, 1995; Rajan, 1998), which is inversely related to bandwidth. After defining the CF and CF-threshold for the excitatory/inhibitory area, the frequency bandwidth at levels from 10 to 60 dB above the excitatory/inhibitory CF-threshold were measured. The excitatory/ inhibitory CF was divided by this bandwidth to obtain a Q value for excitation/inhibition at the appropriate level. Two analyses were done with respect to ERA bandwidths. The first was based on measurements of the total frequency bandwidth of the ERA. In cells with two lobes of responses (e.g. Figs 3d,f, 4d), at each level above CF threshold frequency bandwidths of both lobes were included in calculation of total ERA bandwidth. A later analysis was confined to the bandwidth of only the CF-lobe of the ERA, i.e. only this lobe in cells with dual-lobe ERAs, and only the single (CF) lobe in cells with single-lobe ERAs. In many dual-lobe cells there was clear separation between the two lobes (e.g. Fig. 3d,f) allowing unambiguous measurement of the bandwidth of the ERA encompassed by only the CF lobe. In other cells, the two lobes were continuous (at high intensities). In many cases, it was still possible to make unambiguous determinations of bandwidth limits of the CF lobe of the ERA because the region of continuity consisted of weak responses discontinuously adjacent to regions with much stronger responses in each of the lobes. In some cases such demarcations were not as clear; in such cases, the boundary of the CF lobe ERA at high intensities was determined by linear extrapolation of the slope of the line fitting the boundaries at lower intensities. At least four low-level points were used to determine this line which was then extrapolated to higher intensities to demarcate the boundary between the CF lobe ERA and the second lobe ERA in cells with dual-lobe ERAs.

Intensity Response Functions

Intensity response functions, consisting of recordings of the number of action potentials (APs) in response to each of a number of different levels of a stimulus, were obtained using as stimuli a tone at CF and a broad-band (white noise) stimulus. For each such intensity function, the number of action potentials (APs) in response to each of a number of different levels starting from a level below the visually determined threshold, and increasing systematically in 5 or 10 dB steps up to 80–85 dB SPL. A full set of stimulus intensities in a function defined the intensity response function for that cell for that stimulus. Data were stored in the computer and a printout obtained online. These intensity response functions were classified as to whether they were monotonotonic or non-monotonotonic (Fig. 5d). Non-monotonotonic was defined when responses at high levels decreased to 50% or less of the maximum response (Rajan, 1988). For the CF stimulus intensity response functions were classed only into these two groups since all cells in this study had to be tone responsive to be included. For the white noise stimulus, an additional class was the category of cells that did not respond to the stimulus at all (‘non-responsive cells’).
Some aspects of this data or related data have been reported previously (Rajan, 1998).

**Results**

**Test and Control Databases**

Test single cortical neurons were obtained from six animals with small but statistically significant receptor organ damage, produced some months prior to these experiments, over some restricted and well-defined frequency range in the animal’s hearing range. Figure 1 presents three examples of the hearing sensitivity, measured using the frequency-specific CAP of the auditory nerve. CAP thresholds in each animal are normalized to a large normative database (Rajan et al., 1991, 1993) so that the frequency-specific CAP threshold is expressed as the difference (the hearing loss) from the normative mean CAP threshold at that frequency. In all three cases, as in all test animals, loss in hearing sensitivity was (i) small but statistically significant (hearing losses never more than 25 dB > normal sensitivity), and (ii) restricted to some frequencies between 5 and 24 kHz. Outside this range the animals had normal hearing sensitivity.

The test neurons were from primary auditory cortex (A1) contralateral to the damaged cochlea, and had a CF within the range with cochlear hearing losses. Data were obtained from 36 such A1 cells (hereafter termed ‘test’ cells). Control data were obtained from 21 animals with bilateral normal cochlear hearing sensitivity, tested concurrently with the test animals, from 141 cells (‘control’ cells) with CF at a frequency within similar frequency ranges as the test cells. As noted elsewhere (Rajan, 1998) neurons in test and control animals were obtained from A1 regions that, in the absence of the hearing losses in the test animals, would have very similar response characteristics and interactions.

**Loss of Functionally Defined Surround Inhibition Can Occur Without a Change in the Incidence of Functionally Defined In-field Inhibition**

In Control A1 neurons surround inhibitory areas, when present,
were continuous with in-field inhibitory areas. Examples of control cells possessing both forms of inhibition or only in-field inhibition are shown in Figure 2a,b respectively (and see Fig. 3a–c). Almost equal numbers of control A1 neurons possessed both in-field and surround inhibition (Fig. 2c; Control, open bars), or only in-field inhibition (Fig. 2c; Control, black bars), and only one cell did not possess any inhibition. In test cells (Fig. 3d–f), there was a marked decrease in the proportion possessing both forms of inhibition (Fig. 2c; Test, open bars) and an increase in the proportion possessing only in-field inhibition (Fig. 2c; Test, black bars); two test cells did not possess any inhibition. As reported elsewhere (Rajan, 1998), the proportion of test cells with surround inhibition was significantly lower than the proportion of control cells with surround inhibition ($\chi^2 = 23.63, df = 1, P < 0.001$). However, the proportion of Test cells possessing in-field inhibition did not differ from the total proportion of control cells with such in-field inhibition regardless of whether they also possessed surround inhibition ($\chi^2 = 0.295, df = 1, P > 0.5$).

Definition of suppressive areas with respect to the ERA, by the criteria used here (see Materials and Methods), was not affected by the criterion for defining suppression. This is illustrated in Figure 3 in examples of control cells possessing both surround and in-field inhibition (Fig. 3a,b; Control ‘both’ cells) or only in-field inhibition (Fig. 3c; Control ‘in-field only’ cells), and examples of test cells (Fig. 3d–f) which possessed only in-field inhibition. In most A1 cells there was a sharp boundary between little/no suppression and total suppression; consequently definition of suppression areas as in-field relative to the ERA or additionally being surround was basically independent of the criterion.

Comprehensive data for this report were obtained from 29 test cells possessing only in-field inhibition, 66 control (normal) cells with surround and in-field inhibition (control ‘both’) cells, and 69 cells with only in-field inhibition (control ‘in-field only’) cells. In the case of the test cells, almost equal numbers of cells were obtained from cats with noise-induced hearing losses and cats with idiopathic hearing losses.

**Loss of Functionally Defined Surround Inhibition is not Associated with a Decrease in the Total Size of Functionally Defined Inhibitory Areas**

As a first test of whether loss of surround inhibition in test cells entailed an overall decrease in inhibition it was determined if there was a decrease in the total extent of inhibition. The size of total inhibitory areas (including surround inhibition when that was also present) was measured at levels from 10 to 60 dB above inhibitory CF-threshold, using the $Q$ metric which is inversely related to the size (Sutter and Schreiner, 1995; Rajan, 1998). $Q$ values (Fig. 4a) in test cells did not differ significantly from values in control ‘in-field only’ cells (two-way repeated measures ANOVA; Groups comparison: $F = 0.229, P = 0.635$), and the
systematic increase in bandwidths (decrease in $Q$) with level was similar in both groups (Level: $F = 25.6, P = 0.001$; Group × Level interaction: $F = 1.245, P = 0.28$). Exactly similar effects were found in comparing $Q$ values in test cells to values in control ‘both’ cells (two-way repeated measures ANOVA; Groups comparison: $F = 1.274, P = 0.262$; Level: $F = 22.27, P = 0.001$; Group × Level interaction: $F = 1.318, P = 0.255$). Despite loss of surround inhibition in test cells, the total extent of inhibition was not different from that defined, using exactly similar criteria, in either group of control cells, even those possessing surround inhibition additional to in-field inhibition (control ‘both’ cells). Finally, there were no significant differences between the two control subgroups (two-way repeated-measures ANOVA; Groups comparison: $F = 3.654, P = 0.06$; Level: $F = 97.23, P < 0.001$; Group × Level interaction: $F = 1.986, P = 0.08$); in both groups, inhibitory area bandwidths declined with level in the same systematic manner.

Thus, in neither population of cells with only functionally defined in-field inhibition were inhibitory areas significantly different in size, at any level, from areas in cells with functionally defined surround inhibition additional to in-field inhibition. The fact that inhibitory areas were similar in all three groups suggests that the gain of afferent inhibition, which determines the extent of the inhibitory areas, is remarkably constant, and is maintained even after receptor organ damage. Further evidence in support of this proposition was found in an examination of the excitatory response strength in the test cells compared to the control populations.

**Loss of Functionally Defined Surround Inhibition does not Alter the Strength of Excitation**

A simple overall decrease in inhibition, to produce the observed loss of surround inhibition, increases excitatory response strength in the test cells (Chapman and Stone, 1997). This was assessed by measuring the maximum response at the excitatory CF (Fig. 4b). Across all three groups there were statistically significant differences (one-way ANOVA; $F = 4.21, P < 0.05$). However, post hoc analyses revealed that the difference lay between the two control groups. Although the mean value for test cells was slightly higher than that for control ‘both’ cells, the difference was not significant (Student’s $t$-test: $t = 1.467, df = 93, P_{t \text{,ailed}} > 0.05$). Similarly, the value for test cells was not significantly different from that for control ‘in-field only’ cells (Student’s $t$-test: $t = 0.95, df = 96, P_{t \text{,ailed}} > 0.1$). The absence of a
significant increase in excitatory response strength in test cells compared to control ‘both’ cells is not consistent with a simple decrease in inhibitory gain. Since direct pharmacological blockade of inhibition (thereby reducing or abolishing inhibitory gain) increases the excitatory response strength of central auditory neurons (Martin and Dickson, 1983; Müller and Scheich, 1988; Caspary et al., 1991; Vater et al., 1992; Yang et al., 1992; Palombi and Caspary, 1996), this lack of increased excitatory strength indicates there is no decrease in inhibitory gain in test cells. The two control groups differed significantly (Student’s t-test; t = 2.86, df = 133, P_{1-tailed} < 0.005), with relatively weaker responses in control ‘both’ cells than in control ‘in-field only’ cells.

In the context of the results above on the size of functionally defined inhibitory areas, the result from the test group carries an implication about the strength of inhibition. The conditioning (masker-probe) technique used in this study to functionally define inhibitory areas does not allow direct measurement of the strength of inhibition. However, the criteria used to define inhibitory areas were the same in control cells and test cells (see Materials and Methods) and, as shown in Figure 3, definition of inhibitory areas relative to the ERA was basically unaffected by suppression criteria ranging from 50% suppression of probe responses, to 100% suppression, to the statistical criterion generally used here. Further, as demonstrated here, the strength of excitatory responses to CF tones were not different between the test and control ‘in-field only’ groups. The proportions were reversed in the ‘Control Both’ group where non-monotonic intensity response functions were much more common. Incidence of cells with monotonic and non-monotonic intensity functions, and non-responsive cells, to a broad band (white noise) stimulus in the three groups. The relative proportions of the three response types was similar between test and control ‘in-field only’ groups and were both different from the ‘Control Both’ group where a greater proportion of cells were unresponsive to the white noise stimulus or had non-monotonic intensity response functions.

### Loss or Absence of Functionally Defined Surround Inhibition Leads to an Increase in the Size of Excitatory Areas and Unmasking of New Response Lobes

A general decrease in inhibition should increase bandwidths of excitatory response areas (ERAs) in test cells. The size of ERAs (Fig. 4a) was calculated, using the Q metric, at levels from 10 to 60 dB above the excitatory CF-threshold. Q values in test and control ‘in-field only’ cells were not significantly different (two-way repeated measures ANOVA: Groups comparison: $F = 1.608, P = 0.208$). As with inhibitory areas, the systematic increase in excitatory bandwidths with level was similar in both groups (Level: $F = 97.5, P < 0.001$; Group × Level interaction: $F =$...
In contrast, when test and control ‘both’ cells were compared, there were significant group and level differences and interactions between the two factors (two-way repeated-measures ANOVA; Groups comparison: $F = 37.582, P < 0.001$; Level: $F = 7.104, P < 0.001$; Group × Level interaction: $F = 14.319, P < 0.001$). ERA bandwidths in test cells broadened with level whereas in control ‘both’ cells they narrowed (Fig. 4c), particularly from 30 to 60 dB above CF threshold.

Although this result is consistent with a general decrease in inhibition, it has to be interpreted in the light of data (Martin and Dickson, 1983; Müller and Scheich, 1988; Caspary et al., 1991; Vater et al., 1992; Yang et al., 1992; Palombi and Caspary, 1996) that in-field inhibition alone does not constrain the size of auditory ERAs and showing, or modelling, a need for surround inhibition for this effect in the auditory system, as in other sensory systems (Janig et al., 1977, 1979; Dykes et al., 1984; Ramoa et al., 1988; Alloway et al., 1989; Kaneko and Hicks, 1990; Oka and Hicks, 1990; Alloway and Burton, 1991). Hence the difference between test and control ‘both’ cells could be due to specific loss of surround inhibition in test cells rather than a general decrease in inhibition.

Evidence supporting this proposition was found in the unmasking of new response lobes in test cells. I have shown previously (Rajan, 1998) that in the total test group there is a marked increase in the proportion of cells with dual response lobes (Figs 5d, f, 4d) compared to the proportion in the total population of normal cells. Now, the incidence of dual response lobes in test cells was compared to the incidence of such lobes in the control ‘in-field only’ group (Fig. 4e), with all other ERA types being treated as a single class. The incidence in the test group was significantly higher ($\chi^2 = 5.46, df = 1, P < 0.025$). The fact that these cells, with in-field inhibition only, had a higher proportion of dual-lobed excitatory areas than did control in-field inhibition only cells indicates an unmasking of new response lobes in test cells. The incidence of dual response lobes was also significantly higher in the test group than in the control ‘both’ group ($\chi^2 = 35.98, df = 1, P < 0.001$). Interestingly, the incidence of dual response lobes did not differ significantly between the two control subgroups ($\chi^2 = 1.81, df = 1, P > 0.1$). However, the incidence in both groups was low and the small numbers may not have allowed detection of significance in the greater incidence of dual lobes in the control ‘in-field only’ group (−19%) than in the control ‘both’ group (−10%). In contrast, in the test group here the incidence was −40%, and 50% in the entire (larger) group of test cells (Rajan, 1998).

**Loss of Functionally Defined Surround Inhibition Causes Cells to Revert to the ‘Native’ State of Normal Cells with only Functionally Defined In-field Inhibition**

The above-noted increased incidence of dual response lobes in test cells differentiated them from the control ‘in-field only’ cells, which they resembled in respect of all other data examined to date. The similarity between these groups in other respects was confirmed by comparison of other response properties believed to be shaped by surround inhibition, lacking in both these groups of cells.

The first analysis here was done on ERA bandwidths again, but only on bandwidths of the CF-lobe of the ERA (see Materials and Methods). When CF lobe $Q$ values in test and control ‘both’ cells were compared (Fig. 5a), there significant differences in bandwidths (two-way repeated-measures ANOVA; Groups comparison: $F = 33.13, P < 0.001$; $Q$ values increased with level in control ‘both’ cells but decreased with level in test cells (Level: $F = 6.69, P < 0.001$; Group × Level interaction: $F = 15.09, P < 0.001$). In contrast, when test and control ‘in-field only’ groups were compared, there were no significant differences (two-way repeated-measures ANOVA; Groups comparison: $F = 0.09, P = 0.76$) and the level-dependent pattern of decreasing bandwidths was similar and significant in both groups (Level: $F = 108.28, P < 0.001$; Group × Level interaction: $F = 1.11, P = 0.36$). Finally, the two control subsets differed significantly in the bandwidths and level-dependent bandwidth changes (two-way repeated-measures ANOVA; Groups comparison: $F = 74.6, P < 0.001$; Level: $F = 16.12, P < 0.001$; Group × Level interaction: $F = 33.86, P < 0.001$).

The second analysis was of the incidence of another type of ERA, called circumscribed ERAs (Fig. 5b, and see Fig. 3a, b) as they were constrained both in intensity and frequency terms, and have been suggested to be constrained by the action of inhibition (Vater et al., 1992; Yang et al., 1992). It has been shown elsewhere (Rajan, 1998) that this ERA type is significantly less prevalent in the test cells compared to the total normal population, and it was suggested there that the loss of functionally defined surround inhibition in test cells was responsible for the lower incidence of this ERA type. Here the incidence of this ERA type in the three groups of this study (Fig. 5c) was examined, with all other ERA types treated as a single class. The incidence of this ERA type in test and control ‘in-field only’ groups did not differ significantly ($\chi^2 = 2.04, df = 1, P > 0.1$), but in both was significantly lower than in the control ‘both’ group (versus test group: $\chi^2 = 48.75, df = 1, P < 0.001$; versus control ‘in-field only’ group: $\chi^2 = 79.24, df = 1, P < 0.001$). Thus, the test and control ‘in-field only’ group, both possessing in-field but not surround inhibition, were similar in the relative absence of constrained ERAs, whereas this ERA type was very common in cells possessing both types of afferent inhibition.

The lower incidence of constrained ERAs in test and control ‘in-field only’ cells compared to control ‘both’ cells shows that both the former groups now also respond to high-level stimuli from within the ERA, unlike the control ‘both’ cells which were constrained in intensity. This was confirmed by examining the response function to varying levels of a CF stimulus, with functions classed as being monotonic or non-monotonic according to the shape of the function (Fig. 5d). In the auditory system, inhibition has been shown (Vater et al., 1992; Yang et al., 1992; Pollak and Park, 1993) to be involved in producing non-monotonic intensity response functions to high-level tonal stimuli unlike the purely monotonic functions in cochlear neurones which are not influenced by inhibition. The incidence of these two intensity response function classes in the test group and both control groups is shown in Figure 5e. There is a marked difference in the distribution in the control ‘both’ group, on the one hand, and in test and control ‘in-field only’ groups on the other. The latter two groups did not differ in the incidence of monotonic versus non-monotonic response functions ($\chi^2 = 7.3, df = 1, P > 0.1$), with a much higher proportion of monotonic functions. In contrast, the distribution was significantly reversed in control ‘both’ cells compared to either test cells ($\chi^2 = 51.99, df = 1, P < 0.0001$) or control ‘in-field only’ cells ($\chi^2 = 52.77, df = 1, P < 0.0001$).

The final analysis was carried out on responses to a broadband stimulus, here a white noise stimulus, again examined in intensity response functions. In addition to the two above classes of intensity response functions, a further class consisted of cells that did not respond at all to this broad band stimulus. As illustrated in Figure 5f, again there was a marked difference in
the distribution of response function classes between the control 'both' group versus either the test group or the control 'in-field only' group. The latter two groups did not differ in the incidence of the three response classes ($\chi^2 = 2.76, df = 2, P > 0.1$); in both groups most response functions were monotonic, with much lower numbers of non-monotonic functions or unresponsive cells. The distribution was markedly and significantly different in the control 'both' group compared to either the test group ($\chi^2 = 66.75, df = 2, P < 0.0001$) or the control 'in-field only' group ($\chi^2 = 84.85, df = 2, P < 0.0001$).

Generally, the results show that with loss of surround inhibition in test cells, these cells were less likely to be constrained in the frequency and intensity domains to narrow-band and broad-band stimuli than normal (control) cells which still possessed surround inhibition additional to in-field inhibition. As important, test cells, with a loss of functionally defined surround inhibition but with in-field inhibition, had responses that were very similar to those in normal (control) cells that also only possessed in-field inhibition. These data suggest that test cells reverted to a 'native' state characteristic of normal cells possessing only in-field inhibition (i.e. the control 'in-field inhibition only' cells). Except for the previously noted greater incidence of secondary lobes in the test cells compared to the control 'in-field only' cells, responses in these two groups were otherwise very similar.

Discussion

In this study, test A1 cells, receiving input from cochlear regions with small receptor organ damage, had no functionally defined surround inhibition. Consistent with a loss of inhibition, ERAs in test cells were significantly larger at all levels, except near threshold, than in control cells which received both in-field and surround inhibition (control 'both' cells). Even when analysis was restricted to the CF lobe of the ERA, suprathreshold ERAs in test cells were larger than in control 'both' cells. Test cells were also less likely to be constrained in responding at high levels to a within-ERA (CF) stimulus, or to a broad-band (white noise) stimulus, than control 'both' cells. Finally, unmasking of new responses in test cells was also evidenced in the much greater incidence of dual response lobes compared to either control subgroup.

However, other test results were not consistent with a simple decrease in overall inhibition. First, there was no significant difference in excitatory response strength compared to control cells. Second, level-dependent sizes of total inhibitory areas in test cells had only in-field and surround inhibition whereas test cells had only in-field only' group. Finally, inhibition in test cells did not appear to be weaker. Inhibitory areas were defined as the frequency-intensity space causing a criterion suppression (statistically significant at $\alpha = 0.05$) in responses to an excitatory probe. The facts that the excitatory response strength had not changed in test cells and that, with the same suppression criterion, sizes of total inhibitory areas at all levels in test and control cells were not different, are inconsistent with a simple overall decrease in inhibition.

Locus of Changes in Inhibition

The present study does not directly differentiate cortical from subcortical effects. Forward masking, used here to define functional inhibition, can, for example, produce suppression from within the response area (but not outside) in neurons at the receptor organ (Harris and Dallos, 1979). However, in the forward masking paradigm described herein a delay of 50 ms was used between the end of the masker and the start of the probe. With this delay, receptor level effects are markedly small or absent (Harris and Dallos, 1979). This does not preclude contributions from other subcortical sites where forward masking effects also occur (e.g. thalamus (Schreiner, 1981); midbrain (Zhang et al., 1990); medulla (Rhode and Greenberg, 1994)]. Detailed discussions of the relative contributions of subcortical and cortical mechanisms to forward masking in A1 have been presented previously by others (Calford and Semple, 1995; Brosch and Schreiner, 1997). Based on the pattern of some A1 effects (Calford and Semple, 1995) and on the strength of inhibition in A1 versus subcortical sites (Brosch and Schreiner, 1997), these studies concluded that, for forward masking delays similar or even slightly shorter than that used here, the major locus of effects observed in A1 is in cortex. In this regard, as shown here (Fig. 3), suppression areas in test cells (as in control cells) were very similar whether suppression was defined by a criterion of 100% or 50% suppression of probe responses or by the statistical criterion used as the standard. Thus, as reported by Brosch and Schreiner (Brosch and Schreiner, 1997) forward-masking suppression in A1 cells is very strong (even with a 50 ms masker-probe delay as used here) and this contrasts against subcortical effects (Schreiner, 1981; Brosch and Schreiner, 1997).

Additionally, this study and a previous one (Rajan, 1998) show a very strong correlation between loss of surround inhibition and broadening of ERAs, and a lower likelihood of constraint in responding to high-level CF stimuli or to a broad-band stimulus. These are effects exactly predictable from studies, in auditory and somatosensory CNS, using pharmacological agents to block local inhibition (Martin and Dickson, 1985; Dykes et al., 1984; Alloway and Burton, 1986, 1991; Müller and Scheich, 1988; Ramoa et al., 1988; Alloway et al., 1989; Kaneko and Hicks, 1990; Oka and Hicks, 1990; Caspary et al., 1991; Yang et al., 1992; Palombi and Caspary, 1996). This also makes it likely that local cortical processes must contribute significantly to the effects observed here to allow the observed strong correlation between loss of surround inhibition, defined functionally with forward masking, and A1 responses in the manner predicted from the pharmacological studies.

Thus it appears most likely that these results obtained with a forward masking delay of 50 ms reflect local cortical effects, though subcortical effects may have contributed to the general trend.

Selective Loss of the Surround Inhibitory Component of Afferent Inhibition after Receptor Organ Damage

Almost all aspects of the results in test A1 cells may be explained by a selective loss of only surround inhibition, rather than a generalized loss or decrease in inhibition. This explanation depends on treating afferent inhibition not as a single inhibitory input, as in the most common model of receptive field structure: the difference-of-Gaussians model (Rodieck, 1965; Dawis et al., 1984; Hawken and Parker, 1987; Chapman and Stone, 1966). Instead, I propose that surround inhibition is present only at the flanks of the ERA, where it is an additional afferent inhibitory input co-extensive with excitatory and (in-field) inhibitory inputs. The ERA 'core', where surround inhibition is modelled as absent, consists of excitatory inputs and only in-field afferent inhibition. In the ERA flank regions, the additional afferent inhibition component of surround inhibition suppresses the co-extensive excitatory and in-field inhibitory inputs in these
regions. This model, coupled with a selective loss of surround inhibition without a general decrease in all inhibition, is able to account for almost all the effects observed here, as detailed below.

Surround inhibition is believed to constrain ER As (Janig et al., 1977, 1979; Suga and Manabe, 1982; Martin and Dickson, 1983; Dykes et al., 1984; Suga and Tsuzuki, 1985; Alloway and Burton, 1986, 1991; Müller and Scheich, 1988; Ramoa et al., 1988; Alloway et al., 1989; Kaneko and Hicks, 1990; Oka and Hicks, 1990; Caspary et al., 1991; Vater et al., 1992; Yang et al., 1992; Suga, 1995; Palombi and Caspary, 1996; Rajan, 1998), and thereby constrain and shape responses to broad-band stimuli (Suga and Manabe, 1982; Martin and Dickson, 1983; Suga and Tsuzuki, 1985; Müller and Scheich, 1987, 1988; Yang et al., 1992; Suga, 1995; Calford and Semple, 1995; Brosch and Schreiner, 1997; Rajan, 1998). Loss of surround inhibition can explain the unmasking of new excitatory inputs in test cells in which ER As were broader than in control ‘both’ cells from which they were distinguished, in terms of afferent inhibition, by the absence of functionally defined surround inhibition. Broadening of ER As was not simply due to unmasking of new response lobes as it was also found when considering only the CF lobe of the ERA. Further, the low incidence of circumscribed ER As and of constrained responses to broad-band stimuli was similar in test and control ‘in-field only’ cells, which also do not possess surround inhibition, but in both groups was significantly lower than in control ‘both’ cells, which possess surround inhibition. Selective effects on surround inhibition can also explain the lower incidence of constrained response functions (non-monotonicity at suprathreshold levels) to narrow band (CF) stimuli in test and control ‘in-field only’ cells. At the cochlea, at high stimulus levels there is spread of activity across the receptor array, which may activate surround inhibition in cortical cells possessing this mechanism, and reduce responses at high stimulus levels compared to responses at low levels. Thus, this model can well account for unmasking of new excitatory inputs, including new lobes, and maintains the role of surround inhibition in constraining response functions to broad-band and high-level narrow-band (CF) stimuli.

Selective loss of surround inhibition in this model can also explain why the total extent of afferent inhibitory areas remained constant whether A1 neurons received in-field inhibition alone or surround inhibition as well. In the model surround inhibition when present in the ERA flanks is co-extensive with in-field inputs. In cells receiving only in-field inhibition, the flank excitatory and in-field inhibitory inputs are not suppressed by surround inhibition as they are in cells receiving surround inhibition additional to in-field inhibition. Thus, inhibitory area sizes in both groups of cells are likely to be similar, but in cells receiving only in-field inhibition ER As would be broader as excitation dominates over inhibition in the ERA flanks (cf. control ‘in-field only’ cells versus control ‘both’ cells).

The model does not immediately explain the result that excitatory response strength in test cells was intermediate and not significantly different from that for either control subgroup, whereas the control subgroups differed by a small but significant amount. The latter difference might suggest that surround inhibition shapes suprathreshold responses to within-RF stimuli, because of stimulus spread across the receptor array at high stimulus levels. However, this then does not explain the similarity in excitatory response strength between test and control ‘both’ cells, which possessed surround inhibition additional to in-field inhibition. In-field inhibition appears to control response strength within the ERA in auditory and somatosensory systems (Martin and Dickson, 1983; Dykes et al., 1984; Alloway and Burton, 1986; Müller and Scheich, 1988; Alloway et al., 1989; Kaneko and Hicks, 1990; Oka and Hicks, 1990; Alloway and Burton, 1991; Caspary et al., 1991; Yang et al., 1992; Palombi et al., 1996), and might be the dominant influence shaping excitatory response strength to a within-RF stimulus. In test cells, unmasked in-field inhibitory inputs may exert some control of excitatory strength to CF stimuli, though not as effectively as surround inhibition when the latter was also present. (Note that in the model, surround inhibition is described as being sufficiently strong at the ERA flanks to suppress in-field inhibition from these regions, i.e. surround inhibition is stronger than in-field inhibitory inputs that are unmasked after loss of surround inhibition.) This would fit with the fact that control ‘both’ cells had significantly weaker excitatory responses than control ‘in-field only’ cells and that test cells had a response strength intermediate between the two control groups.

Finally, it is noteworthy that, in many ways, responses of test A1 cells were similar to normal A1 cells possessing only in-field inhibition. The two groups had similar-sized inhibitory areas, similar excitatory response strengths, and, for the CF lobes of ER As, similar-sized excitatory areas. They only differed in the greater prevalence of dual response lobes in the test cells. These results suggest that small receptor organ damage caused test cells to lose surround inhibition and ‘revert’, in many ways, to a native state characterized by the control cells with only in-field inhibition.

**Unmasking of Inhibitory Inputs after Receptor Organ Damage**

The model advanced above predicts that loss of surround inhibition will unmask new inputs, made up of excitatory and (in-field) inhibitory components, from the ERA flanks where both inputs had been previously suppressed by co-extensive surround inhibition. The unmasking of inhibition is consistent with reports in raccoons that an immediate effect of digit amputation is unmasking of inhibition from adjacent digits in cortical (Rasmusson and Turnbull, 1983; Turnbull and Rasmusson, 1990; Rasmusson et al., 1992) and thalamic (Rasmusson et al., 1993) neurons formerly receiving input from the amputated digit. One difference is that in the current study, unmasked inhibitory inputs from ‘off-focus’ regions were not the sole or dominant input – new excitatory inputs from these regions dominated the new inhibitory inputs were in-field to excitation from these regions. This difference may simply reflect time after receptor organ damage when recordings were made – within 1 week in the raccoon studies and >1 month here. In the raccoon studies, unmasked inhibitory inputs were not observed if >1 week was interposed between receptor organ damage and recordings. However, the investigators examined only for obvious and dominant inhibitory effects (such as off responses and inhibition of spontaneous activity); this would not have identified inhibition when excitation was dominant, as with in-field inhibition. So if off-focus excitation started to dominate, the off-focus inhibitory inputs would be subsumed as in-field inhibition and would not be observed. This may also explain the fact that in the raccoon studies, unmasking of inhibitory inputs was only found after loss of all input from a region of the receptor surface (Turnbull and Rasmusson, 1990) and not after partial denervation, whereas the present study found the unmasking after small receptor organ damage. Testing only for
obvious and dominant inhibitory effects may not have revealed in-field inhibition in the raccoon studies.

The stereotyped effects in this study after chronic auditory receptor organ damage contrast with the idiosyncratic effects seen immediately after damage to the receptor organ induced by a loud sound (Calford et al., 1993). Immediate effects on A1 neurons, examined only on ERAs (Calford et al., 1993), ranged from simple changes reflective of receptor organ changes, to complex changes, in different neurons, suggestive of a decrease in inhibition relative to excitation, an increase in inhibition relative to excitation, or differential effects on excitation and inhibition in different parts of the neuron’s ERA. In contrast, the present study shows that the long-term effects are a stereotyped loss of surround inhibition and unmasking of excitatory and in-field inhibitory responses. It is possible that longer-term effects may reflect dominant local (cortical) effects, while short-term effects, which include effects simply reflecting receptor organ changes (Calford et al., 1993), may include effects at many levels of the neuraxis.

**Selective Loss of Surround Inhibition and Plasticity of Cortical Maps**

Anatomical studies show that receptor organ damage can result in loss of cortical inhibition (Hendry and Jones, 1986; Hendry and Kennedy, 1986; Warren et al., 1989; Akhtar and Land, 1991; Hendry et al., 1990; Skangiel-Kramská et al., 1994; Micheva and Beaulieu, 1995). These studies do not differentiate between different functional forms of inhibition and it is difficult to know whether such changes directly relate to the selective effects on functionally defined surround inhibition reported here. Further, the anatomical studies have shown changes following receptor organ damage that caused, or was of the sort known to cause, plasticity of topographic maps. In contrast, Test animals of this study did not show plasticity of the A1 frequency map of the cochlea (Rajan, 1998) in cortical regions receiving input from the damaged regions of the receptor surface (the cortical regions in which the test cells of this study were located). Thus, small receptor organ damage of this study may not produce the anatomical changes described above. It has been suggested (Müller and Scheich, 1988; Alloway et al., 1989) that surround and in-field inhibition may be differentiated by the latter acting through somatic hyperpolarization (Rose 1977; Müller and Scheich, 1988) and performing a ‘divisive’ operation (Rose, 1977; Alloway et al., 1989), and the former acting ‘subtractively’ (Alloway et al., 1989) at dendrites (Müller and Scheich, 1988; Alloway et al., 1989). This may be achieved through different types of GABA receptors (Kaneko and Hicks, 1990; Oka and Hicks, 1990) with GABA<sub>B</sub> receptors involved in distal effects and GABA<sub>A</sub> receptors in somatic effects, consistent with topological differences in GABA receptor distribution (Connors et al., 1988). These differences may allow anatomical visualization of whether small receptor organ damage leads to selective loss of functionally defined surround inhibition.

Loss of surround inhibition appears to cause plasticity of cortical suprathreshold responses (current study) without causing plasticity of topographic maps (Rajan, 1998) defined from the most sensitive inputs. To date such map plasticity in cortex has been demonstrated only after loss of all afferent input (surround and in-field inhibition, and excitation) from a region of the receptor surface (Rasmussen 1982; Merzenich et al., 1983, 1984; Kelahan and Doetsch, 1984; Robertson and Irvine, 1989; Kaas et al., 1990; Heinen and Skavenski, 1991; Pons et al., 1991; Chino et al., 1992; Gilbert and Wiesel, 1992; Rajan et al., 1993; Schwaber et al., 1993; Darian-Smith and Gilbert, 1995; Schmid et al., 1996). In contrast, small auditory receptor organ damage has been shown not to produce A1 map plasticity in two separate studies (Rajan, 1998; Rajan and Irvine, 1998), one on the animals providing the test cells of this study. Although test cells still possessed in-field inhibition, it is difficult to see how loss of in-field inhibition alone could produce plasticity of topographic maps defined from the most sensitive inputs. It seems more plausible to suggest that plasticity of sensory cortical maps of the receptor surface occurs only after loss of all inhibition and dominant excitation. Thus, if loss of inhibition plays a critical permissive role in plasticity of sensory cortical maps, this requirement may not have been met in the cortical regions in test animals from which the test cells used in this study were derived.

**Notes**

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Address correspondence to R. Rajan, Department of Physiology, Monash University, VIC 3800, Australia. Email: ramesh.rajan@med.monash.edu.au.

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