Differences in Somatosensory Processing in S1 Barrel Cortex between Normal and Monoamine Oxidase A Knockout (Tg8) Adult Mice

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Spatio-temporal processing of whisker information was analysed in vivo for single neurons in D2 barrel columns of S1 cortex in Tg8 mutant mice, which lack barrels. Findings were compared with normal C3H mice of the same genetic background. The topographical organization of functional columns was similar in Tg8 and normal mice. Response magnitudes (RMs) to D2 principal whisker deflections in D2 columns for Tg8 were similar to normals for layers I–III and layer IV cells but short latency responses (>10 ms post-stimulus) were twice the magnitude of normal mice. The surrounding whiskers D1 and D3 yielded smaller RMs in layer IV of mutants than normal mice whereas RMs in layers I–III were equipotent (P > 0.5). Modal latencies were shorter in Tg8 mice in all layers. Latency distributions for whisker D2 responses in both laminae were bimodal in normal mice, peaking at 6–8 ms post-stimulus, but unimodal in Tg8 mice in both laminae, peaking at 6–8 ms. Hence, despite an absence of barrels, segregation of columns is enhanced in layer IV and sensory processing is faster in layers I–IV compared with normal mice. This contrasts with adenylyl cyclase knockout mice where both an absence of barrels and enhanced surrounding whisker responses have been observed. These findings suggest that factors other than barrels and clustering of thalamo-cortical terminals define receptive field geometry.

Introduction

Following its discovery in 1970 (Woolsey and Van der Loos, 1970), the ‘barrel’ cortex of rodents has achieved increasing popularity as a model for uncovering the functional roles of thalamo-cortical and intra-cortical networks in the processing of somatosensory information (Simons, 1978; White, 1978, 1979; Armstrong-James and Fox, 1987; Armstrong-James et al., 1991, 1992, 1993; Agmon and Connors, 1992; Diamond et al., 1992a,b; Welker et al., 1993; Melzer et al., 1994; Armstrong-James, 1995; Diamond, 1995; Kyriazi et al., 1996, 1998). The principle attraction of barrel cortex lies in its barrel-shaped cell aggregates defining columns of thalamo-cortical terminals in layer IV (Woolsey and Van der Loos, 1970), which replicate the pattern of whiskers precisely. Barrel cortex has provided a test-bed for dissecting the way in which thalamus and cortex functionally interact to process whisker information both radially within functional columns and tangentially for inter-columnar integration (Armstrong-James and Fox, 1987; Bernardo et al., 1990; Armstrong-James et al., 1991, 1992, 1993; Kossut, 1992a,b; Armstrong-James, 1995; Hoeflinger et al., 1995; Kim and Ebner, 1999; Kleinfeld et al., 1999). Barrels also reflect the profound segregation of sensory inflow from individual whiskers (Woolsey and Van der Loos, 1970; Killackey, 1973; Jensen and Killackey, 1987) such that they were originally suggested by Woolsey and Van der Loos (Woolsey and Van der Loos, 1970) to be structural manifestations of discrete functional columns (Mountcastle, 1957). This role for barrels received support from a recent study on the mutant mouse barrelless (Welker et al., 1996), which is devoid of the adenylyl cyclase A gene (Abdel-Majid et al., 1998). The lack of barrels in this mouse correlates with larger and poorly centred receptive fields and a broader spread of thalamo-cortical axon arbor in layer IV when compared with normal mice. In the present study the hypothesis that barrels constitute an anatomical organization which accentuates columnar segregation is tested further for generality in another mutant mouse (Tg8), which also fails to exhibit barrels by virtue of deletion of the monoamine oxidase A gene (Cases et al., 1995, 1996). An excess of serotonin during the critical period of barrel formation is suggested to prevent barrel formation. This is deduced from the observation that administration of parachlorophenylalanine in Tg8 pups, which inhibits serotonin synthesis, reinstates formation of barrels in S1, whereas inhibition of catecholamine synthesis does not (Cases et al., 1996). The same authors have found that tgm8 mice are more aggressive than wild-type mice on the same C3H background but are physically similar. However, tgm8 mice possess somewhat shorter snouts and are less physically adept. They exhibit normal brain sizes and gross structure.

Surprisingly, in this mouse, unlike barrelless, we find an enhanced segregation in layer IV of whisker receptive fields, despite an absence of barrels, and faster responses to whisker stimulation when compared with normal mice.

Materials and Methods

Two groups of mice were used in this study. C3H normal mice (normals or NOR) and monoamine oxidase A (MAOA) knockout mice on a C3H background (Tg8) (Cases et al., 1995, 1996). The transgenic Tg8 mouse fails to generate barrels in S1 cortex, whereas barreloids in the ventro-posterior medial thalamic somatosensory relay are present, although less distinct than normal (unpublished data). For the experiments reported here the experimental procedures were identical for the two species. Studies were performed on a total of 32 male mice (C3H controls, n = 17, weight 27.32 ± 0.35 g; Tg8, n = 15, weight 28.56 ± 0.66 g). Post-natal ages for each group ranged between 9 and 13 weeks. For neurophysiology mice were anaesthetized by the i.p. route with urethane (10% solution in distilled water, 2 mg/g body wt). The animal was then placed in a headholder (Kucera, 1970) on a stereotaxic apparatus and exposed to a continuous transnasal flow of 100% oxygen. The skin was incised near the midline and a 5 × 5 mm square area of postero-medial barrel cortex was exposed by left-sided craniotomy using a small electric drill. The dura mater was left intact and continuously irrigated. Body temperature was maintained at 36–37°C by a rectal thermistor controlled heating pad (Harvard plc, UK). Under these conditions the animals did not whisk, no eye blink reflex could be elicited and pinching of the hind foot reluctantly evoked a withdrawal reflex. If an animal showed any alteration of these signs, an extra 10% of the initial dose of urethane was given i.p.

Cell Sampling

Recordings were made with carbon fibre microelectrodes (Armstrong-James and Millar, 1979) with conical tips. Since the mutant tgm8 mice have no barrels, for each group of animals (Tg8 and controls) penetrations were targeted on cells maximally responsive to whisker D2 and...
exhibiting the shortest latency to that whisker for comparative analysis of receptive properties. Physiological properties only of such cells are described below. Microelectrodes were lowered normal to the dura surface and easily penetrated the dura mater with negligible visible dimpling. Contact with the pia surface was verified through a dissection microscope (Zeiss) and subsequent increased neuronal noise as the cortex was penetrated was detected by a noise clipper. Action potentials were enhanced by bandpass filtering at 0.8–8 kHz using Neurolog spike processing modules (Digitimer, UK) for display on a Gould digital oscilloscope with a built in pre-stimulus display (Gould, USA). Single cells were discriminated by amplitude and duration of negative and positive spike components using an in-house waveform window dual level discriminator. Cells were selected by the same criteria in Tg8 and normal mice. Action potential heights had to exceed five times the baseline noise in the absence of spikes and only cells with initial negative action potentials were used. All cells exhibited some spontaneous activity, exceeding 1 spike/20 s. When the first two cells encountered responded maximally to D2 then all cells down to a depth of at least 350 μm (deep layer IV) were investigated for responses to all five whiskers. Penetrations ranged from 1.2 to 2.0 mm caudal to the bregma, 3.5 to 4.0 mm lateral to the midline and 0 to 0.45 mm in depth from the surface of the cortex for recording cells in layers I–IV (Welker et al., 1993, 1996), which is where D2 columns were consistently found in normal and Tg8 animals (see Results). A calibrated 3-dimensional stepping motor microdrive (Narishige, Japan) which had an operational accuracy of 10 μm in each direction was used for electrode displacement.

**Whisker Stimulation**

To deflect individual whiskers on the right side of the face, a 0.5 mm wide flat probe, bonded to a piezoelectric ceramic wafer stimulator, was positioned just below the shaft of the whisker, ~4 mm from the skin. The wafer was deflected by a computer-gated trapezoid pulse. The stimulus was delivered just below the shaft of the whisker, bonded to a piezoelectric ceramic wafer stimulator, was positioned just below the shaft of the whisker. A calibrated 3-dimensional stepping motor microdrive (Narishige, Japan) which had an operational accuracy of 10 μm in each direction was used for electrode displacement.

**Data Analysis**

Using a CED-1401 processor and Spike 2 software (Cambridge Electronic Design, Cambridge, UK) with an in-house program, post-stimulus time histograms (PSTH) and latency histograms were generated on-line. Latency histograms were constructed from the times of occurrence of the first spike generated after each stimulus (modal latencies). Null responses were excluded, as were responses which failed to generate clear modal peaks within 5 ms bins for surrounding whiskers or 1 ms for D2 central whisker stimulation. The response magnitude to a single whisker was calculated as the total number of spikes obtained 300 ms post-stimulus to 50 whisker deflections minus the equivalent period of spontaneous activity derived from the mean value immediately prior to each stimulus series (Armstrong-James and Fox, 1987). All data were stored on disk at a resolution of 10 μs on a Pentium PC for on-line or off-line processing and statistical analysis.

**Centre–Surround Contrast**

The summed response to the surrounding D row whiskers, D1 and D3, is taken here to serve as an index of the immediate neighbouring receptive field input relative to a D2-centred cell in the barrel cortex. The centre–surround contrast is defined as (D1 + D3)/D2, where values for D1, D2 and D3 are expressed as response magnitudes (see above).

**Histology**

At the end of recording sessions small lesions were made at sites in the cortex from which recordings were made, using a 1.5 kA negative current applied for 5 s between the microelectrode and earth. Subsequently, the brain was removed from the cranium and fixed in 4% paraformaldehyde solution. Serial coronal sections (60 μm thick) were cut and mounted on gelatinized slides and air dried. Sections were stained in cresyl violet or with the cytochrome oxidase technique for visualization of lesioned sites and barrels. Cells were ascribed to two groups: either to the SG layer, (layers I–III) or to layer IV. The barrels span layer IV and to some extent penetrate lower layer III and contain the clustered terminals originating from the nucleus ventroposterior medialis (VPm), the principal thalamic relay nucleus (Killackey, 1973; Keller et al., 1985; Lu and Lin, 1993). Barrels are separated tangentially by a ring of tightly packed neurons forming the barrel wall. In common with our earlier studies on mouse barrel cortex (Welker et al., 1993, 1996) we found that the barrel junction between the SG layer and layer IV extended over 250–300 μm below the pial surface. The layer IV/Va junction was at 400–450 μm. Accordingly, cell populations were divided into two groups, SG layer cells being those found within 270 μm of the pial surface and layer IV cells those located between 270 and 425 μm from the pial surface. Although Tg8 mice exhibit no distinct barrels, laminar delineation of layers I–IV appeared normal, confirming a previous study (Cases et al., 1996). Therefore, for the Tg8 animals similar depths were used for separating cells into the two layer classification.

**Results**

The principal objectives of this study were to discern whether receptive fields and temporal responses of barrel cortex cells differed in normal and Tg8 mice and whether any differences might reflect known anatomical differences.

**Topographical Organization of Barrel Cortex in Tg8 Mice**

Since the barrel cortex of normal mice is topographically organized as a map of the whisker pad in the postero-medial barrel subfield (Woolsey and Van der Loos, 1970), the procedure we used for finding the D2 ‘column’ in normal and in Tg8 mice was as follows. The first microelectrode penetration in any experiment was placed ~3.5 mm lateral and 1.5 mm caudal to the bregma. If this revealed successive cells maximally responsive to the E2 whisker the next penetration would be moved 200 μm further lateral to the expected site of the D2 column in a normal mouse. If cells were maximally responsive to D1, C2 or D3, for example, then the next penetration would be moved rostral, caudal or medial, respectively, to the expected site of the D2 column. This procedure was invariably successful in Tg8 as well as normal mice and the D2 column was found within 200–300 μm of an adjacent D1, D3, C2 or E2 column in Tg8 and normal mice. Tg8 mice therefore appear to exhibit a similar topographical organization to the barrel cortex of normal mice, at least for the D1, D2, D3, C2 and E2 columns.

**Column Validity**

When the first two cells encountered in a penetration responded maximally to D2 then all cells encountered to a depth of at least 350 μm were investigated for responses to all five whiskers. To establish whether columns were specifically centred on only one whisker, cells were counted which failed to respond maximally to D2. In normal mice we found that 93 cells out of a total of 100 studied responded maximally to the D2 whisker; the remaining seven responded maximally to an adjacent whisker. In Tg8 mice 138 responded maximally to the D2 whisker of 140 cells studied. These findings suggest that radial columnar segregation is as good in Tg8 mice as in normal mice with the same genetic background.

The findings which follow reflect cells sampled in complete penetrations through layers I–IV only in the D2 column. Within the D2 column analysis was restricted to responses from cells located at depths of 0–425 μm spanning the first four cortical layers (see Materials and Methods). Since barrels are...
absent in Tg8 animals the D2 column was defined physiologically by cells responsive at minimal latencies and maximal response magnitudes in layer IV to deflection of whisker D2. For the findings presented below cells were allocated for analysis separately within the SG layer (0–270µm depths) or within granular layer IV (>270 to <425µm). A total of 100 cells in normal (NOR) mice and 139 cells in Tg8 mice were studied for response to 50 stimuli applied to the centre-receptive ‘principal’ D2 whisker and to each of the adjacent surrounding receptive field (SRF) whiskers D1, D3, E2 and C2. Incompletely tested cells were rejected. In normal mice 42 and 58 cells were classified as being located in layers I–III (SG layer) and layer IV, respectively. For Tg8 mutant mice 90 and 49 cells were classified as being located in layers I–III and layer IV, respectively.

Response to the D2 Principal Whisker

The mean response magnitude was defined by the number of spikes generated over 300 ms post-stimulus in response to 50 stimuli applied to each whisker corrected for spontaneous activity (see Material and Methods). The receptive field configurations for the cell populations in the NOR and Tg8 groups are displayed by layer in Figure 1A, in terms of the mean response magnitude to D2, D1 and D3 whisker inputs. In the SG layer mean response magnitudes to the D2 whisker were equivalent for NOR and Tg8 mice (64.0 ± 5.2 and 59.5 ± 2.8, respectively), differences not being significant \( P > 0.5 \) (Mann–Whitney U-test). Response magnitudes to D2 for layer IV cells in the NOR and Tg8 groups also did not differ (51.8 ± 3.2 and 53.6 ± 2.9, respectively; \( P = 0.5 \)). Values for spontaneous activity were assessed for each cell immediately prior to whisker stimulation to enable the contribution to responses to be compensated for by subtraction from raw response magnitudes.

The findings for spontaneous activity are compared in each subset of layers for control and Tg8 mice in Figure 1B, where it can be seen that spontaneous activity in Tg8 mice was, on average, one-third to one-quarter that for normal mice. Differences between normal and Tg8 mice were highly significant \( P < 0.0001 \) in each case,
**D1 and D3 Whisker Responses**

Responses to stimulation of whiskers D1 and D3 were much smaller than to the principal D2 whisker in both Tg8 and normal mice in both the SG layer and layer IV (Fig. 1A). In Tg8 mice mean response magnitudes did not differ for deflection of whisker D1 or D3 for cells in either layer (Fig. 1A; P > 0.05, Wilcoxon signed rank test (WSR)). In NOR mice D1 and D3 responses were also equivalent in magnitude in layer IV (P > 0.05), although responses to D1 were marginally larger than to D3 in the SG layer in normals (P = 0.04). On comparing NOR and Tg8 animals, responses of layer IV cells to both the D1 and the D3 whiskers were significantly smaller in Tg8 compared with NOR animals (D1, P > 0.001; D3, P > 0.05, MWU) (Fig. 1A). In contrast, response magnitudes for SG layer neurons were not significantly different between NOR and Tg8 animals for whiskers D1 and D3.

**Centre–Surround Contrast**

The relative contributions of immediately surrounding whiskers as against the principal whisker to responses of a cell gives an index of how well the principal whisker defines the centre of the receptive field. The average of the summed responses to the D row surrounding whiskers (D1 + D3 response magnitudes for individual cells) is used here as an index of the immediate neighbouring in-row surrounding receptive field input to a D2-centred cell in the barrel cortex.

D row surrounds of layer IV cells contributed much less to the receptive field in Tg8 mice compared with normal mice (ΣD1 and D3, 16.3 ± 2.4, n = 98, and 28.8 ± 2.8, n = 116, respectively; P > 0.001, MWU). However, for SG layer cells the difference between Tg8 and normal mice for the surrounding D row response was not significant (24.6 ± 2.2, n = 180, and 26.2 ± 2.8, n = 84, respectively; P > 0.5). Comparing layer IV with SG layer cells, the mean surrounding D row responses were significantly smaller in layer IV for Tg8 mice (ΣD1 and D3, 16.3 ± 2.4, n = 98, and 24.6 ± 2.2, n = 180; P > 0.001, MWU), but were equivalent for SG and layer IV neurons in normal mice (ΣD1 and D3, 26.2 ± 2.8, n = 84, and 28.8 ± 2.8, n = 116; P > 0.5).

The values for mean centre-surround contrast for cells in different layers in normal and Tg8 animals are shown in Figure 2 (note that increasing centre-surround contrast is indicated by smaller values). In the SG layer centre-surround contrast was entirely similar for normal and Tg8 mice, the difference not being significant (0.41 ± 0.05 and 0.41 ± 0.04, respectively; P > 0.9, MWU). However, layer IV cells in Tg8 animals exhibited much higher centre-surround contrast compared with normal mice (0.31 ± 0.04 and 0.49 ± 0.05, respectively; P > 0.001) and hence considerably better defined receptive field centres.

The underlying reasons for differences in layer IV are shown in Figure 3, where the distribution of response magnitudes for D row surrounds are shown for cells in the different layers (left and centre columns). Distributions are skewed towards smaller responses in all instances (Fig. 3A,B,D,E) but the cumulative distributions for layer IV cells in Tg8 mice exhibited an abnormal proportion of null and small responses compared with normal mice (c.f. Fig. 3D,E). This is most clearly shown by comparison of the cumulative distributions for layer IV cells (Fig. 3F, lower right). However, for SG cells the cumulative distributions for D1/D3 surround responses were found to be entirely similar for Tg8 and normal animals (Fig. 3C).

**Temporal Configuration of Responses: PSTH Distributions**

Figure 4 shows examples of the types of PSTHs generated in response to deflection of the principal whisker, D2, for cells in normal and Tg8 mice. For normal mice similar patterns of responses were encountered in layers I–III and in layer IV. Responses ranged from multimodal (Fig. 4A–C) to unimodal, with the earliest discharges peaking at 5–20 ms post-stimulus. This early peak constituted the solitary discharge of unimodal responses (Fig. 4D), but was present in all other types of cells encountered. Later clusters of spikes were generated at various post-stimulus intervals, most of which terminated within 100–200 ms post-stimulus, although some extended to 300 ms post-stimulus. Although similar types of responses could be found in layers I–III and IV, discharges at 10–100 ms post-stimulus were less vigorous in layer IV on average than of cells in layers I–III.

Responses of cells in Tg8 mice exhibited less variation in form and hence were more stereotyped than were responses of normal mice. Nearly all responses peaked within 15–20 ms post-stimulus in all layers tested (I–III and IV) and almost invariably failed to show any specific secondary discharge. Instead, discharge rates declined progressively over various periods to 300 ms post-stimulus. These characteristics were similar for layers I–III and layer IV cells.

Absolute response magnitudes do not show how sequential components of the response may differ for populations of cells within and between groups. To allow this analysis the original PSTHs were condensed into six sequential epochs (0–10, 10–20, 20–50, 50–100, 100–200 and 200–300 ms) and mean values for distributions calculated. This was done for responses to each whisker for each cell in all cell populations described in the previous section. An analysis in this way enables discrimination of differences between early and later components of the response and gives insights into sequential relays within the column (Armstrong-James et al., 1992). These epoch PSTHs were averaged for all cells in the specified layers for comparisons between and within groups.
The findings for the D2 (principal) whisker are shown in Figure 5A–D for SG layer and layer IV cells in the two groups of animals. Several differences are evident when comparisons are made between these series of histograms.

Firstly, the magnitude of the shortest latency (>10 ms) discharges is much greater for Tg8 mice than for the normal controls. This was the case for both the SG layers (Tg8, 21.0 ± 2.2 spikes/50 stimuli; normal, 10.7 ± 1.6 spikes/50 stimuli; P > 0.01, MWU) and layer IV cells (Tg8, 20.4 ± 2.5 spikes/50 stimuli; normal, 11.6 ± 1.4 spikes/50 stimuli; P > 0.02, MWU). Reflecting these findings, for Tg8 mice the temporal distribution of PSTH components is dominated by shorter latency components (>20 ms post-stimulus) independently of layer, whereas for normal mice the principal whisker response peaks at intermediate latencies (20–50 ms).

In summary, when comparing mutants with controls the temporal distribution of the response to the D2 whisker differs consistently between Tg8 and control animals regardless of layer, but when comparing the different layers within individual species similarities in response profiles predominate. This suggests fundamental differences between the two groups of mice in sequential synaptic processing of D2 whisker information from layer IV to layer II in the D2 column.

**D2 Principal Whisker Response Latencies**

PSTHs and epoch analysis allow an examination of the time course of complete responses evoked by the principal and SRF whiskers. However, to analyse response onset times, latency histograms were generated from the first spike to each stimulus of a given whisker. For control mice mean latencies for SG cells to the D2 principal whisker were significantly different from layer IV cells and on average 2.3 ms later (12.4 ± 5.6 and 10.1 ± 4.3 ms, respectively; P = 0.03, MWU), in accordance with synaptic transmission from layer IV to SG cortex. In contrast, mean latencies for cell populations in the SG layer and layer IV were not significantly different in Tg8 animals (9.4 ± 2.5 and 9.4 ± 2.5 ms, respectively; P > 0.05, MWU).

**Differences in Modal Latencies for Surrounding and Principal Whiskers (Fig. 7)**

Figure 7 shows the mean modal latencies for responses to
surrounding and principal whiskers. For D2 responses >96% of cells had measurable modal latencies. For surrounds only 14 (Tg8, C2/E2 whiskers) to 53% (NOR, layer IV, D1/D3) of cells had measurable latencies since latencies were not available for null responses or for very small responses (see response distributions in Fig. 3). Hence, our findings are biased towards measurable responses only. Measurable surrounding whisker latencies were universally much later than responses to the principal whisker. This was the case in all layers for both normal and Tg8 mice. Measurable latencies were not significantly different between surrounding in-row (D1/D3) and in-arc (C2/E2) whiskers in any layer for either Tg8 or normal mice. Differences in mean measurable latencies between principal and surrounding whiskers varied from ∼25 to 30 ms.

Modal Latency Distributions
Figure 8 compares modal latency distributions for cells responding to the D2 whisker for the separate cell populations in the SG layer and layer IV in the control and Tg8 groups. For control animals distributions were bimodal in both the SG layer and layer IV, suggesting two classes of cells. In both SG cortex and layer IV one population of cells fired at a modal latency value of 6–8 ms post-stimulus. A secondary population fired predominantly at around 10–12 ms in layer IV and at 12–14 ms in the SG layer. In contrast, in Tg8 mice the latency distribution was unimodal at the earlier latency of 6–8 ms for both the SG layer and layer IV, with no evidence of a distinct group of cells firing at longer latencies. For Tg8 mice latency distributions were very similar for SG layer and layer IV cells.

Discussion
Evidence is sparse for the effect of serotonin and norepinephrine on receptive fields in S1 cortex when sustained at elevated levels over the developmental period to maturity. Iontophoresis of serotonin onto adult cortical neurons reduces sensory driven responses in rat S1 (Waterhouse et al., 1986) and reduces the contrast between driven and spontaneous activity in V1 cortex (Waterhouse et al., 1990). Conversely, norepinephrine ionto-
phoresis enhanced visually evoked discharge of V1 cortical neurons (Waterhouse et al., 1990). In hamsters a single dose of 5,7-dihydroxytryptamine at birth causes hyper-innervation of the superior colliculus by serotoninergic fibres (Ke et al., 1999) and irregularities in the retinotectal projection. However, receptive fields of tectal neurons appear normal despite the receptive fields of retinotectal afferents being enlarged. Together these studies suggest that sustained or transient elevation of extracellular serotonin at maturity acts to contain the receptive field size of cortical and tectal cells and this may go some way to explaining the reduced magnitudes of SRFs seen in Tg8 mice in the present study. However, developmental failure of thalamo-cortical fibres to cluster into barrels and barrel development per se may also contribute to these differences, and these are discussed below.

The most significant finding from the present study is that cells located in the SG layer (layers I–III) of barrel cortex of mice deficient in the MAOA gene (Tg8 mice) respond at considerably shorter latencies to deflection of the principal whisker for their column compared with normal animals of the same genetic background (Figs 4, 6 and 8). In normal C3H mice the latencies of cells in the SG layer to stimulation of the principal whisker were 2.3 ms later, on average, to cells responding to the same stimulus in layer IV, where the main body of thalamo-cortical afferents terminates. These inter-laminar differences are similar to those found for the rat barrel cortex (Armstrong-James et al., 1992) and indicate, on average, two or three synaptic relays between layers (see below) (Agmon and Connors, 1991). However, in the Tg8 mice mean modal latencies for SG cells were within 1 ms of the value for cells located in layer IV. In the mouse thalamo-cortical slice preparation Agmon and Connors found that differences in latencies between SG layer and layer IV cells ranged from 0.5 to 2 ms for current sinks (Agmon and Connors, 1992), which constituted ~90% of their sample. EPSPs of SG layer cells in the same experiments were most commonly evaluated as disynaptic to VPm electrical stimulation. It is not known how many SG cells above barrels receive monosynaptic inputs from VPm.

**Thalamo-cortical Transmission**

In previous studies on rats and mice spike discharges generated
in barrels which occur within 8 ms of the stimulus onset to their principal whisker have been attributed conservatively to monosynaptic relay by thalamo-cortical afferents from VPm (Armstrong-James and Fox, 1987; Armstrong-James and Callahan, 1991; Welker et al., 1993, 1996). If in the present study it is assumed that cells firing at modal latencies ≤ 8 ms constitute populations with significant monosynaptic input from VPm, then 50% of SG cells and 58% of layer IV cells in Tg8 mice match those criteria (Fig. 8). This compares with 37 and 50% of cells for layers I–III and IV, respectively, with modal latencies of ≤ 8 ms in the control mice.

Fast radial transmission through cortex in Tg8 mutants was paralleled by the finding that responses to the principal whisker in both the SG layer and layer IV (Fig. 4) peaked within 10 ms post-stimulus in both layers and then declined to low values by 50 ms. Conversely, in normal mice the peak response to the principal whisker occurred later (10–20 ms) in both the SG layer and in layer IV, which agrees with earlier studies on normal mice with a different (BALB/C) background (Welker et al., 1993) and with albino rats (Armstrong-James et al., 1992, 1993).

**Origins of Accelerated Processing in Tg8 mice**

One possibility for faster radial transmission in MAOA-deficient mice is that their barrel fusions could be due to continued exuberant growth of these fibres in development (Vitalis et al., 1998). In normal mice the radial invasion of barrel cortex by specific thalamo-cortical afferents from VPm is restricted to barrels in layer IV at maturity, avoiding the septal zones that sculp barrels (Killackey, 1973). Cells in the lower region of layer III in normal mice make synapses with thalamo-cortical inputs through their basal dendrites (White, 1978). In Tg8 mice there are clearly some thalamo-cortical terminals invading layer III and some even penetrating layer II (Cases et al., 1996) (unpublished observations). However, comparisons of labeling of thalamo-cortical afferents of different laminae from barrel cortex coronal sections revealed by orthograde transport of biotinylated dex-
transient deposited in VPm suggest very similar laminar distributions of thalamo-cortical afferent arbors in Tg8 and normal mice (Cases et al., 1996). The available evidence to date on thalamo-cortical afferent distributions do not, therefore, explain the origin of the shorter latencies for Tg8 mice in superficial cortex. One possibility is that thalamo-cortical afferents have more profuse arbor ramifications in the Tg8 mutants which are not evident from the dextran staining used for these studies. This could perhaps be resolved by single thalamo-cortical axon reconstructions.

A second possibility for accelerated transmission is that first relay intra-cortical axons from layer IV pyramidal/spiny stellate cells innervate upper layers of the column more profusely, allowing faster EPSP rise times and hence shortened latencies, although an increase in response magnitude might also be expected to result. Indeed, although overall the response magnitudes to principal whisker deflections were equivalent for Tg8 and normal mice in our study, short latency (>10 ms post-stimulus) components of PSTHs specifically were twice the magnitude found for normal mice, followed by reduced longer latency components (Fig. 4). This reconfigured temporal distribution of the principal response in Tg8 mice is in line with a more potent generation of short latency spikes in layer IV secondarily driving more effective disinhibitory effects in layer IV and the upper layers in mice (Agmon and Connors, 1992; Agmon et al., 1996). To resolve this notion, a specific investigation of VPm relay in Tg8 mice would be required to see if it is abnormally powerful or fast.

A Role for Differential NMDA and AMPA Receptor Transmission?

In our study the fastest principal whisker responses in layer IV for all mice occurred at similar latencies to those in the rat, namely at 6–8 ms, where a clear mode occurred for the distributions of latencies. In rat and mouse barrel cortex sensory transmission is entirely dependent upon AMPA and NMDA receptors (Armstrong-James et al., 1993; Gil and Amitai, 1996a,b). In those studies the fastest responses to principal whisker deflection also formed a compact mode of spikes at 6–8 ms post-stimulus, which in the rat are entirely attributable to AMPA receptor activation (Armstrong-James et al., 1993). In mouse in vivo thalamo-cortical slice preparations the early latency EPSPs of single layer IV (barrel) neurons generated by electrical stimulation of VPm are similarly attributable to AMPA receptor activation (Gil and Amitai, 1996b). In both cases the longer latency responses to the same stimulus are dependent upon NMDA receptor activation. To recall the findings in the present study: 50% of SG cells fired at latencies of >8 ms in the mutant mice compared with 37% of SG cells in the normal animals; for layer IV, 58% of cells fired first within 8 ms in the mutants, compared with slightly less than 50% in normal animals. These figures indicate only onset latencies and give a higher figure than for proportions of early discharges (derived from PSTHs). Nonetheless, these analyses of modal latencies would suggest that substantially more cells in layers 1–IV of Tg8 mutant animals achieve significant monosynaptic engagement with thalamo-cortical afferents operating through AMPA receptors compared with normal mice.

A suitable origin for surplus short latency responses in the Tg8 animals, therefore, may be excessive AMPA receptor participation in thalamo-cortical responses. This hypothesis could receive confirmation from quantitative iontophoretic techniques, to separate AMPA from NMDA components of responses (Armstrong-James et al., 1993; Salt, 1987), and immunohistochemical techniques. It should be appreciated, however, that all cell populations in both types of mice generated most of their spikes at latencies >10 ms post-stimulus. Many of the later discharges may arise alternatively from the prolonged open times of NMDA receptors (Collingridge and Bliss, 1987) or reiterative relay within the column (Douglas et al., 1995; Armstrong-James, 1995). In this respect prolonged activation of cells to brief (3 ms) principal whisker deflection were similar in mutants and controls.

Stimulus Repetition Rates

It is known that stimulus repetition rate (SRR) affects response magnitudes of S1 cortical neurons in vivo (Armstrong-James, 1975; Armstrong-James and George, 1988). In those studies stimulation rates were varied between 0.2 and 5 stimuli/s and response magnitudes progressively decreased with increasing rates. Our conclusion was that a rate of 1 stimulus/s, commonly used in other studies, could usefully be employed as a standard for comparison of S1 cortical responses in vivo and between species. Using a SRR of 1 stimulus/s here the detectable response to the surrounding, but not principal, whiskers, extended to 300 ms and it is possible that part of the response was compromised by this SRR. However, examination of peri-stimulus time histograms revealed only rare occurrences of spikes during the 50 ms period prior to stimulation unless spontaneous activity levels were high.

Formation of Surrounding Receptive Fields

In contrast to the observations on responses to principal whiskers, responses to surrounding whiskers occurred at exceptionally long latencies. For layer IV no significant short latency events (>10 ms post-stimulus) were evoked by surrounding whisker stimulus in Tg8 or normal mice, in contrast to principal whisker inputs. In Tg8 mice, but not normal mice, a small proportion of surrounding responses in the 5G layer above barrels generated early discharges at response levels of >5% of those to the principal whisker. The latter may arise from aberrant divergent thalamo-cortical afferents, not seen in normal mice and most likely located within layer III. SRF magnitudes (D row surrounds) for normal C3H mice in this study were similar to those found previously (Wellker et al., 1993) in BALB-C mice and exhibited equivalent long latencies of 40–45 ms with no significant differences for different layers (Fig. 7). Differences between latencies to principal and surrounding D row whiskers were 30 and 28 ms in layer IV and the 5G layer, respectively, in normal mice. For Tg8 mice cells fired on average some 22–26 ms later to surrounding D row than to principal whiskers in layers 1–IV. In normal rat barrel cortex, using urethane anaesthesia as in the present study, substantial latency differences between principal and surrounding whiskers also occur, being 7–13 ms longer in latency to adjacent SRF whiskers, and responses to the latter are entirely dependent upon column-to-column relay of principal whisker information (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991; Fox, 1994; Armstrong-James, 1995). Whether a similar cortical origin for the same SRFs in D2 barrels of rats holds for the mice studied here remains to be proven; in rats sedated with fentanyl and paralysed, for example, all surround responses in layer IV and some in layers II–III are found to be relayed through the thalamus to the cortex (Goldreich et al., 1999). However, under identical conditions of anaesthesia to those used here VPm cells fire at modal latencies differing by only 2–3 ms for principal and
adjacent surround inputs (Armstrong-James and Callahan, 1991), which would not account for the very large latency differences seen here for the same whiskers in barrel cells. Hence, the most likely origin for SI cortical SRFs in both the Tg8 and normal mice remains column-to-column relay of principal whisker information, as in rats under similar experimental conditions. Finally, it is conceivable that the posterior nucleus (medial division) of the thalamus (POm) could contribute to SRFs in barrel cortex. However, in the rat POm neurons have been shown to derive their small long latency large receptive fields by reflective relay from barrel cortex (Diamond et al., 1992a,b). If subsequent re-entrant relay to cortex is successful then their contribution to background depolarization rather than directly to SRFs could be significant.

Short latency responses to principal whisker deflection were more powerful in Tg8 mice in all layers (I–IV) than in normal mice (Fig. 4). Consequently, in Tg8 mice one might have expected more powerful transfer of tangential information to generate SRFs in adjacent columns, assuming that local inhibition between adjacent columns did not differ from normal mice. However, SRFs in layer IV were of lower magnitude in Tg8 mice (Fig. 1). This would suggest that tangential relay between columns in layer IV is less effective in Tg8 mice than in normal mice. For SG cells SRFs were of equivalent magnitude in Tg8 and normal mice (Fig. 2), but still would have been expected to be more powerful in Tg8 mice if cortico-cortical transmission was similar. The fact that spontaneous activity was compromised in Tg8 mice might suggest a lower level of background depolarization on average in cortical neurons, whether due to a lower incidence of afferent excitation or increased intra-cortical inhibition. The outcome is likely to compromise intra-cortical relay, which is dependent upon adequate NMDA receptor activation requiring cell depolarization (Armstrong-James et al., 1993) and hence weakens SRFs. In addition, deficient SRFs in Tg8 mice might also arise from enhanced column-to-column inhibition, confining column-to-column relay in layer IV in the mutants and less so in upper (SG) layers. In normal mice inter-columnar relays appear overwhelmingly excitatory in mouse barrel columns, with inhibitory relay only being local to the column in layers I–IV (Aroniadou-Anderjaska and Keller, 1996).

Our observations seem to rule out excessive tangential spread of functional VPm afferent arbors denoting single whiskers across more than the equivalent of a barrel width within individual barrel columns in either normal or Tg8 mice. If this were the case, latencies to surrounds would be expected to be quite close to principal whisker latencies. Secondly, since in Tg8 mice SRF inputs generated weaker responses than was the case for normal control mice, our findings suggest even greater functional targeting of thalamo-cortical afferents to layer IV in Tg8 mutants than in normal mice, and thus enhanced columnar segregation. The confined receptive field profiles in layer IV of Tg8 mice contrast markedly with findings on the barrelless (brl) mutant mouse discovered by Welker, which was found to have larger receptive fields in layer IV than normal mice, a feature which correlated with much broader spreads of thalamo-cortical arbors in layer IV (Welker et al., 1996). In brl mice excess overlap of thalamo-cortical afferents in the absence of barrels appears sufficient to explain poorly centred receptive fields. In our Tg8 animals, which are similarly without barrels, quite different processes must generate their heightened centre-surround contrast of receptive fields. Hence, the presence of barrels cannot be construed as an absolute requirement for generating cortical circuitry for sharpening the acuity of receptive fields and other, yet to be defined, mechanisms within development are able to achieve this outcome, at least in Tg8 mice.

Notes
We thank Drs Egbert Welker and Patricia Gaspar for helpful discussions on these experiments. This work was supported by European Union Grant (Biomed 2) BMH4 CT-97-2412.

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