Whisker maps of neuronal subclasses of the rat ventral posterior medial thalamus, identified by whole-cell voltage recording and morphological reconstruction

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Whole-cell voltage recordings were made in vivo in the ventral posterior medial nucleus (VPM) of the thalamus in urethane-anaesthetised young (postnatal day 16–24) rats. Receptive fields (RFs) on the whisker pad were mapped for 31 neurones, and 10 cells were recovered for morphological reconstruction of their dendritic arbors. Most VPM neurones had antagonistic subthreshold RFs that could be divided into excitatory and inhibitory whiskers. VPM cells comprised different classes, the most frequently occurring being single-whisker excitation (SWE) and multi-whisker excitation (MWE) cells. In SWE cells (36 % of VPM neurones), only principal whisker (PW) deflection evoked an EPSP and was followed by a single action potential (AP) or remained subthreshold. The depolarisation was terminated by a large, delayed IPSP. A stimulus evoked on average 0.74 ± 0.46 APs (mean ± s.d.) with short latency (8.1 ± 1.0 ms) and small temporal scatter (0.31 ± 0.23 ms dispersion of 50 % of the first APs). In MWE cells (29 % of VPM neurones), deflection of several whiskers evoked EPSPs. PW responses were either subthreshold EPSPs or consisted of an EPSP followed by one or several APs (0.96 ± 0.99 APs per stimulus). AP responses were often associated with putative low-threshold calcium-dependent regenerative potentials and were followed by a small delayed IPSP. AP responses had a longer latency (12.3 ± 2.6 ms) and larger temporal scatter (2.5 ± 1.6 ms) than responses of SWE cells. MWE cells had a lower input resistance than SWE cells. The elongation of dendritic arbors along the representation fields of rows and arcs in VPM barreloids was weakly correlated with the subthreshold RF elongation along whisker rows and arcs, respectively. Evoked EPSP–AP responses exhibited a sharper directional tuning than subthreshold EPSPs, which in turn exhibited a sharper directional tuning than IPSPs. In conclusion, we document two main classes of VPM neurones. SWE cells responded with a precisely timed single AP to the deflection of the PW. In contrast, MWE cell RFs were more broadly tuned and the temporally dispersed multiple AP responses of these cells represented the degree of collective deflection of the PW and several adjacent whiskers.

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The rodent whisker-to-barrel cortex pathway has a number of properties that facilitate the experimental analysis of this sensory representation (Jones & Diamond, 1995). The electrical signals generated by deflection of individual facial whiskers are processed in discrete neuronal aggregates in the brainstem, thalamus and cortex (Woolsey & Van der Loos, 1970; Jones & Diamond, 1995). Whiskers and whisker follicles are easily accessible, and experimental whisker manipulations have revealed the experience-dependent plasticity of this sensory system (Van der Loos & Woolsey, 1973; Armstrong-James, 1995; Polley et al. 1999). Deflections can be applied to the whisker array in a precise fashion, and the structure of receptive fields (RFs) of whisker-movement-driven neurones can be mapped in a highly quantitative manner (Ito, 1981; Simons, 1983). Whisker-driven thalamic neurones might serve as model neurones with which it may be possible to elucidate how thalamic cells transform the pattern of afferent impulses into a new pattern conveyed to the cortex. The ventral posterior medial nucleus (VPM) of the rat thalamus is the major thalamic target for trigeminal whisker afferents from the contralateral brainstem nuclei, in particular the principal trigeminal nucleus (Lund & Webster, 1967; Erzurumlu & Killackey, 1980). The VPM is also the major source of the whisker-driven input to the barrel cortex and receives corticothalamic feedback excitation. The VPM is a thalamic subnucleus with a strictly topographic organisation (Emmers, 1965; Waite, 1973a). Excitatory inputs from individual vibrissae are relayed by cell clusters termed 'barreloids' (Van der Loos, 1976), each containing approximately 250 neurones (Land et al. 1995). VPM cells
also project axon collaterals to cells in the thalamic reticular nucleus, and the reticular cells inhibit neurones in the VPM. Intrathalamic connections are made mainly or exclusively between VPM and reticular neurones. Neither local inhibitory interneurones in the VPM (Barbaresi et al. 1986) nor recurrent connections between VPM cells have been observed.

Extracellular unit recordings have shown that supra-threshold RFs of VPM neurones are larger and their responses are of a more transient nature than the whisker-evoked responses in the brainstem nuclei (Waite, 1973b; Shipley, 1974; Ito, 1988; Simons & Carvell, 1989). Pharmacological manipulations, lesion studies and cross-correlation analysis of simultaneous dual unit recordings suggest that inhibitory inputs from the reticular thalamic nucleus modify thalamic responses to whisker deflection (Shosaku, 1986; Shosaku et al. 1989; Lee et al. 1994a,b). Recent studies emphasise the large size of VPM neurone suprathreshold RFs (Nicolelis et al. 1993) and the modulation of RF properties by anaesthesia or by the behavioural context (Diamond, 1995; Fanselow & Nicolelis, 1999; Friedberg et al. 1999).

While these studies provide a comprehensive view of the action potential (AP) patterns evoked by whisker movements in VPM neurones, little is known about the synaptic mechanisms that generate these patterns, and few thalamic cells of known RF structure have been identified morphologically (Harris, 1986; Chiaia et al. 1991; Ohara & Havton, 1994).

We have made in vivo whole-cell voltage recordings from morphologically identified VPM neurones to (a) compare quantitatively their sub- and suprathreshold RFs, (b) classify VPM neurones according to their RF structure and their morphology, and (c) identify possible intrinsic cell properties that may contribute to their response patterns. The results indicate that most VPM neurones have an antagonistic subthreshold RF divided into excitatory and inhibitory whiskers. VPM neurones fell into at least two major classes according to their subthreshold RFs, their temporal response profile and their passive properties. These two classes of neurones are likely to contribute differently to the electrical representation of whisker deflections in the rodent cortex.

**METHODS**

**Animals**

Forty Wistar rats of both genders were used. Thirty-five animals were between 16 and 24 days old. The remaining five animals were between 28 and 32 days old; the data collected from the older animals did not differ from the other data and all results were therefore pooled. All morphologically reconstructed cells were from the younger age group.

**Preparation**

Rats were anaesthetised with an i.p. injection of urethane (1–1.5 g kg⁻¹). The animal’s temperature was monitored with a rectal probe and maintained between 36 and 37°C by a thermostatically controlled heating pad. Once anaesthetised, animals were placed into a stereotactic frame and their head fixed with atraumatic earbars and a muzzle clamp. A 1.0 mm hole was drilled in the skull at 2.75 mm lateral and 3 mm posterior of bregma. The dura was removed with a 30 gauge injection needle tip and the exposed cortex was superfused with warm artificial cerebrospinal fluid solution. All experimental procedures were carried out according to the animal welfare guidelines of the Max-Planck Society.

**Anaesthesia**

Depth of anaesthesia was assessed by monitoring pinch withdrawal, eyelid reflex, corneal reflex, respiration rate and vibrissae movements. In the recording conditions chosen here, pinch withdrawal and vibrissae movements were usually absent, but weak eyelid and corneal reflexes could be observed. Respiration rates were usually between 70 and 100 breaths min⁻¹. Occasionally, both vibrissae movements and withdrawal reflexes began to appear during the course of the experiment; in these cases an additional dose of urethane (20% of the initial dose) was given. Taken together, these observations suggest that the depth of anaesthesia varied in our animals around anaesthetic state III-3 (Friedberg et al. 1999). This conclusion is strengthened by observations from whole-cell recordings in cortical neurones, which were made under the same experimental conditions and occasionally also simultaneously with recordings from thalamic neurones. In these recordings, which are known to resemble in frequency content the respective EEG or the electrocorticogram (Creutzfeld, 1993), we rarely observed spindles or a dominance of low-frequency (1–2 Hz) activity, both of which are indicative of deep anaesthesia stage III-4 (Friedberg et al. 1999).

**Whole-cell recording**

In vivo whole-cell voltage recordings were made with long-taper patch pipettes with DC resistances of 4–6 MΩ pulled from borosilicate glass tubing on a Sutter puller in a three-stage pull. Pipettes were filled with (mM): potassium gluconate 130, sodium gluconate 10, Hepes 10, phosphocreatine 10, MgATP 4, Na₂ATP 2, GTP 0.3, NaCl 4 and 0.4 % biocytin, at pH 7.2.

Pipettes were lowered vertically into the VPM. The average depth at which cells were recorded was 4635 ± 136 μm. Pipette tips could become occluded during the passage to this depth. To prevent electrode tip occlusion, high pressure (> 100 kPa (1000 mbar)) was applied to the pipette interior during the vertical lowering. Occasionally, pipettes were advanced through a guide tube made from the tip of a 22 gauge injection needle. Despite these measures, tip occlusion remained a problem and series resistances of thalamic recordings were higher than those of recordings made from cortical cells.

To establish the whole-cell recording configuration we applied a conventional voltage-clamp technique (Blanton et al. 1989; Margrie et al. 2001). Pipettes were advanced into the brain in 2 μm steps while pulsing 8–15 mV steps of 10 ms duration. Positive pressure (2.5–4 kPa (25–40 mbar)) was applied to the pipette while it was being lowered. Positive pressure was removed when an increase in pipette resistance was detected as a sudden decrease in the current pulse amplitude. This usually resulted in a seal...
resistance of 1 GΩ or higher. Occasionally, slight negative pressure was applied to achieve establishment of a giga-ohm seal. The whole-cell configuration was established by applying a slow ramp of negative pressure. Voltage recordings were amplified using an Axoclamp 2B amplifier (Axon instruments, Foster City, USA), filtered at 3 kHz and digitised at 5–20 kHz (ITC-16; Instrutech, New York, USA). Series resistances were between 25–90 MΩ. All electrophysiological data was corrected for a +7 mV junction potential.

**Sensory stimulation and receptive fields**

Before recording, whiskers contralateral to the cortical recording site were trimmed to a length of ~12 mm. Three types of whisker stimuli were delivered: whisker deflection by a hand-held probe, by airpuffs and by a piezoelectric stimulator. An elongated hand-held probe was used for applying hand-held stimuli. Airpuffs were generated from pulses of compressed air, which could be delivered in a computer-controlled way by a picospritzer unit (General Valves, Fairfield, NJ, USA). Airpuffs were applied through a stiff micropipette tip with a 2 mm opening positioned 10–15 mm rostralateral from the whiskers. Under these conditions, the airpuff stimuli deflected 4–8 whiskers in 2–3 whisker rows by up to 2 mm. A piezoelectric bimorph wafer with an attached glass capillary tube served for quantitative single-whisker stimulation (Simons, 1983). Steps elicited by the piezoelectric device had a 10–90% rise time of 0.6 ms. In some experiments the rise time was slowed down to 1 ms with a filter amplifier to reduce ringing. Since responses were not significantly different in these two stimulation conditions, the data were pooled. The deflection point of the whisker was chosen to be 8–10 mm from the base of the vibrissa, and if not otherwise noted the vibrissa was deflected by 1 mm (roughly 6° deflection angle) for 200 ms.

Once a whole-cell voltage recording had been established, the intrinsic properties of the cell were assessed by injection of current steps. The input resistance of the cell was measured at steady state (after 300 ms of current injection) at that voltage (usually around −70 mV), where the smallest amount of inward and outward rectification was apparent. Afterwards, spontaneous activity was sampled over a 20–100 s period. Then the airpuff stimulus was moved across the whisker array and 20 airpuff stimuli were applied at the most effective position. Qualitative RF mapping was performed by deflecting individual whiskers with the hand-held probe. Only cells that gave consistent supra- or suprathreshold responses to both at least one airpuff position and at least one type of hand-held stimulus were considered to be whisker-driven. In whisker-driven cells, quantitative RF mapping was carried out by deflecting individual whiskers with the piezoelectric stimulator 20 times at 1 Hz in a backward (rostral to caudal) direction. Cells for which the identification of the principal (the most effective) whisker (PW) was unambiguous from the hand-mapping procedure, were included in our RF-classification if five whiskers (that is the PW and half of the surrounding whiskers) had been mapped this way. Cells where a principal whisker could not be established by hand mapping entered our RF classification sample only if at least 10 whiskers had been mapped. After mapping 9–18 whiskers, the PW and those surrounding whiskers (SUWs) that evoked the strongest responses were determined. These whiskers were then deflected in four different directions (back, forth, up and down relative to the orientation of the whisker rows) and with varying amplitudes.

**Data analysis**

Various aspects of whisker-evoked responses were quantified within the 100 ms after stimulus onset. The AP count within that time window was classified as the neurone’s suprathreshold response. If a response contained more than one AP within 10 ms, this was classified as a burst. The excitatory response peak (ERP) was defined as the largest membrane depolarisation apparent in the averaged whisker response with the following properties. First, in the averaged response the depolarisation had to exceed 1 mV (measured from the membrane voltage in the 5 ms before the stimulus). Second, events contributing to the peak had to occur within a 10 ms latency window in more than 10% of the stimulation trials. Similarly, hyperpolarisations were classified as inhibitory response peaks (IRPs) if they met the same requirements. In cases where APs were evoked, determination of the ERP amplitude was more complex. Where APs were evoked in less than 50% of the cases, ERP amplitudes were determined as the peak of the postsynaptic potential not including AP amplitude. Where APs were evoked in 50–95% of the cases the ERP amplitude was considered to be equal to the largest postsynaptic potential without an AP. In the few cases where there were no AP failures (n = 4), the ERP amplitude was assumed to equal the largest value of the postsynaptic potential before the onset of the APs plus 5 mV. This stipulation is obviously somewhat arbitrary, but due to the small number of cases it did not strongly affect our statistical assessment of ERP amplitudes.

In many responses we observed a sequence of an ERP and an IRP. For the sake of simplicity, in our RF plots only the first of these peaks (i.e. usually the excitatory one) is depicted. By limiting quantification to the first 100 ms after the stimulus, we excluded the sustained response components from entering the analysis. Although ERPs and IRPs did not represent purely excitatory or inhibitory responses, we refer to them as EPSPs and IPSPs, for the sake of simplicity.

For PSP latency measurements we determined the time point after whisker deflection onset where the postsynaptic potential reached 5% of its peak amplitude. AP latency was measured as the time after whisker deflection onset to the peak of the AP. Unless noted otherwise, we report median values of latencies. The measurement of median latencies leads to higher estimates of latency values, but this measure has the advantage of being independent of the number of measurements and being insensitive to outliers.

To quantify the direction tuning of various response components, a directionality index was calculated as follows: (response in the preferred direction – response in the opposite direction)/ (response in the preferred direction + response in the opposite direction). Thus, values of 1 represent completely directional responses, values of 0 represent completely non-directional responses.

**Histological procedures and reconstruction**

On completion of the physiological recordings, animals were perfused transcardially (when necessary after an additional dose of urethane) with 0.1 M PBS followed by a solution of 4% paraformaldehyde. The brain was removed from the skull and immersed in fixative for at least one more day, after which it was sectioned coronally in 100-μm- or 300-μm-thick slices. Slices were then processed with the avidin–biotin–peroxidase method (Horikawa & Armstrong, 1988) to reveal cell morphology, and mounted on slides using moviol. Subsequently, biocytin-labelled
neurones were reconstructed with the aid of Neurolucida software (Microbrightfield, Colchester, VT, USA) using a Zeiss Axioplan microscope at a magnification of ×1000. Reconstruction resulted in three-dimensional cell morphology. The analysis of the relationship of dendritic field geometry and RF elongation was restricted to two-dimensional coronal views for the following reasons. First, because the geometry of barreloid rows is simple and well established in the coronal plane, whereas it is complex in the anteroposterior axis (Sugitani et al. 1990), and second because cells appeared to be artificially flattened in the anteroposterior axis by the staining and embedding process. The soma diameter was calculated as the mean of the maximal and the minimal soma diameter. To quantify polarisation of the dendritic tree, we determined the largest angular segment free of dendrites in a distance of 30 µm from the soma.

The localisation of cells in the thalamus was assessed directly after the staining and before the embedding procedure. In those relatively freshly cut sections we usually found it possible to delineate the borders of the VPM. The border between the VPM and the posterior medial nucleus (PoM) could usually be delineated by the more transparent appearance of the PoM in transmitted light and the more granular appearance of the VPM in reflected light. Although the majority of recorded cells could not be recovered, we observed in many of the respective brains electrode tracks in the area of the VPM. Specifically, we observed that many cells and electrode tracks were located in the lateral

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**Figure 1. Soma-dendritic morphology and responses of a single-whisker excitation (SWE) cell**

A, schematic representation of the whisker arrangement in the rat’s face. B, left: schematic drawing of cell position in the rat thalamus. Right: coronal view of reconstruction of cell morphology. Both the thalamus and the cell have been rotated such that barreloids representing whisker rows run horizontally and barreloids representing whisker arcs run vertically. D = dorsal, L = lateral. C, two successive responses to principal whisker (PW; whisker D4) deflection. D, two successive responses to surround whisker (SuW; whisker D5) deflection. Onset and offset stimulus artefacts are seen in all records as small deflections. E, subthreshold responses to stimulus onset, dark bars refer to IPSPs, whereas light bars refer to EPSPs. F, action potential responses to stimulus onset. Whisker positions shaded grey in E and F refer to the responses shown in C and D.

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parts of the VPM, and it therefore seems unlikely that our sample of cells contained a substantial number of cells from the PoM.

RESULTS
Results were obtained from 54 whole-cell voltage recordings of whisker-driven VPM thalamic neurones. For 31 of these cells, sub- and suprathreshold RFs were mapped, and 10 neurones were recovered for morphological reconstruction of their dendritic arbors. All identified cells were located in the VPM. The average spontaneous AP activity was $0.037 \text{s}^{-1}$.

Sub- and suprathreshold RFs
Figure 1A and B shows an example of a neurone with the most frequently encountered RF structure. It responded to deflection of its PW with a short-latency (8.7 ms) EPSP-AP sequence followed by a large IPSP (Fig. 1C). Deflection of the eight surrounding whiskers evoked either a large IPSP (Fig. 1D and E) or the cell did not respond. Surrounding-whisker (SuW) deflection did not evoke depolarisation. Both EPSP and IPSP responses to the onset of whisker deflection were substantially stronger than stimulus-offset responses at the end of the deflection.
(i.e. when the whisker moved back into its original position). Other cells shared response properties with the cell illustrated in Fig. 1 and were classified as single-whisker excitation (SWE) neurones, the criterion being that only a single whisker could evoke an EPSP upon deflection. These neurones represent the most frequent cell type recorded (11 out of 31 cells, 36%).

Figure 2 A and B illustrates a second type of cell, responding with either an EPSP followed by one or several APs or only with a subthreshold depolarisation to deflection of its PW (Fig. 2 C). The later part of the depolarisation lasted longer, and in its time course resembled that of low-threshold Ca\(^{2+}\)-APs (Jahnsen & Llinás, 1984; for review see Steriade et al. 1997). The initial depolarisation was usually not followed by a large IPSP as in SWE cells. SuW deflections evoked either an EPSP (Fig. 2 D), IPSPs, or no responses (Fig. 2 E). In this neurone the EPSPs evoked by the most effective SuW deflection (wE1, Fig. 2 E) did not evoke an EPSP large enough to trigger APs (Fig. 2 F), and in the suprathreshold RF of this neurone the SuW excitation was therefore not apparent. The responses to stimulus onset both as EPSP or IPSP were substantially larger than the responses to the stimulus offset. Other cells responding to the deflection of multiple whiskers with excitation shared response properties with the cell illustrated in Fig. 2, and were classified as multiple whisker excitation (MWE) cells. The criterion for a MWE cell was that deflection of more than one whisker evoked EPSPs. MWE cells represent the second most frequently recorded RF type (9 out of 31 cells, 29%).

The recorded cells were classified according to the scheme shown in Fig. 3. Here, cells that responded to multi-whisker stimulation by an airpuff but not to the smaller deflection applied by a piezoelectric stimulator were classified as high-threshold neurones. In addition, we recorded from neurones that responded only with IPSPs. Finally, cells were observed with an inhibitory RF centre, where a single whisker that evoked strong inhibition was surrounded with weak excitatory responses from multiple whiskers (inhibitory centre, IC cells, Fig. 4).

**Average RF maps**

Figure 4 compares the average subthreshold RF maps of the different RF types with their respective suprathreshold RF maps. RFs were aligned according to the PW response. SWE neurones (Fig. 4 A, left) had a prominent initial EPSP in response to PW stimulation, and inhibitory SuWs. The average amplitude of inhibition evoked by SuW deflection along the row (IPSP = \(-2.53\) mV) was significantly larger than the average amplitude evoked along the arc whiskers (IPSP = \(-1.05\) mV, paired \(t\) test, \(P < 0.02\)). The suprathreshold RF was restricted to a single whisker.

MWE neurones depolarised in response to both PW and to SuW stimulation (Fig. 4 B), but their suprathreshold RFs were much smaller (Fig. 4 B, right). Inhibitory whisker responses were observed in most MWE cells, but they are not apparent in the averaged RF due to the dominance of the larger excitatory responses. Indeed, a separation of the RF into excitatory and inhibitory whiskers was observed in most SWE and MWE cells. The majority of neurones (17 out of 20 SWE and MWE cells) exhibited at least one excitatory response and at least one inhibitory response when different whiskers were deflected.

Figure 4 C illustrates the receptive field of IC neurones. A characteristic property of their RF organisation was the relatively small depolarisation evoked by the excitatory SuWs (Fig. 4 C, left). The RFs of all-whisker inhibition (AWI) cells were characterised by large hyperpolarisations induced by deflections of both the PW and SuW (Fig. 4 D). While on average only about half of the cells had

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**Figure 3. Classification and distribution of neurones from the ventral posterior medial nucleus of the thalamus (VPM) according to receptive field (RF) structure**

IC = inhibitory centre; AWI = all-whisker inhibition.
Figure 4. Averaged sub- and suprathreshold receptive field (RF) maps of different VPM neurones

Averaged RF plots for cells of RF types. Prior to averaging, RFs were aligned to the respective PW. Left column: subthreshold responses to stimulus onset. Only the first response peak was considered. Right column: suprathreshold (AP) responses to stimulus onset. A, SWE cell average. B, MWE cell average. C, IC cell average. D, AWI cell average. All AWI cells had asymmetric RFs in which the PW was bordering the whisker array. This asymmetry was preserved here by choosing an asymmetric RF plot and by mirror imaging one of the RFs before averaging.
asymmetric RFs, all 5 AWI cells did. The PW was bordering the whisker array.

**Response amplitudes and RF maps**
Almost all subthreshold RFs comprised several whiskers. For all cells, 15% (on average 1.2 whiskers) of the 8 SuWs evoked excitatory responses (none of the SWE cells and 37% of the MWE cells). For all cells, 40% (3.6 whiskers) of the 8 SuWs evoked inhibitory responses (45% in SWE cells and 38% in MWE cells). Suprathreshold RFs were usually single-whisker RFs; only 4 (13%) out of 31 cells had multi-whisker RFs. For all cells, only 4% (0.3 whiskers) of the 8 SuWs evoked AP responses (no SWE cells and 14% of MWE cells).

Figure 5A and B illustrates quantitatively the properties of PW and SuW responses of neurones with different RF maps. For PW stimulation the initial depolarisations recorded in SWE and MWE cells were of comparable amplitude, whereas the subsequent IPSPs were significantly larger in SWE cells (ANOVA, $P < 0.01$). PW stimulation

**Figure 5. Response amplitudes**
A, average amplitudes of EPSPs, IPSPs, and AP responses for PW deflections. Error bars represent 1 s.d. B, average response amplitudes for the SuW deflections, which evoked the largest response. The values for EPSPs and IPSPs in MWE cells are from different whiskers. C, plot of AP counts evoked by airpuffs versus those evoked by single-whisker PW stimuli. For clarity only SWE and MWE cells are shown.
evoked a comparable number of APs in SWE and MWE cells, but while most SWE cells responded with a single AP in response to a PW stimulus, the AP rates of MWE cells varied across cells (\(F\) test for the ratio of variances, \(P < 0.03\)).

Figure 5C compares AP responses evoked by single-whisker deflection (applied by a piezoelectric device to the PW), with responses evoked by a multi-whisker deflection (applied by an airpuff). For both SWE and MWE cells the multi-whisker deflection was less effective than the single-whisker stimulus. For all SWE and MWE cells, an average of 0.32 APs per multi-whisker stimulus were evoked, significantly less than the 0.62 APs per single-whisker stimulus (paired \(t\) test, \(P < 0.05\)).
Dendritic morphology and subthreshold RFs

Table 1 shows the key morphological parameters of the soma and dendrites of 10 reconstructed cells. A comparison between dendritic morphology and the physiological properties of these cells suggest two types of correlation.

Firstly, the geometry of dendritic arbors appears to reflect the ‘row’ geometry of subthreshold RFs. In coronal sections, the whiskers of a single row are represented as an approximately dorsoventral stack of VPM barreloids. The more dorsally located barreloids represent the more posterior whiskers. They are slightly displaced laterally (Waite, 1973a; Sugitani et al. 1990; Diamond, 1995; Land et al. 1995). Consistent with an overall tendency of RFs to be elongated along whisker rows (e.g. Fig. 4A and B), the axis of maximal field span of 7 dendritic fields of 10 neurones was more closely aligned with the row axis of the barreloids than with the orthogonal axis. Cells for which row neighbours of the PW gave (in absolute terms) stronger responses than arc neighbours of the PW, had a dendritic field elongation consistent with the barreloid row axis (Figs 1B, 2B and 7A). In contrast, neurones whose dendritic elongation was closer to the barreloid arc axis had symmetrical RFs (Fig. 6A), or had RFs where arc neighbours of the PW gave stronger responses than the row neighbours of the PW (Fig. 6D). Secondly, it appears that dendrites are not confined to barreloid borders. One of the remarkably constant features of VPM cells was their maximal dendritic field span, on average 267 µm. This value suggests that the dendrites were not confined to individual barreloids (Waite, 1973a; Sugitani et al. 1990; Land et al. 1995). Barreloids have considerably smaller diameters, in particular along the vertical axis their width is well below 100 µm. Direct evidence for this view is,
significantly longer in MWE cells (Fig. 9). Identical latencies, but the latency of the first AP was less pronounced than the variation of inhibitory latencies of excitatory responses according to RF position (Fig. 9) and the variation of inhibitory responses (Fig. 9) and MWE (Fig. 8). Excitatory responses had shorter latencies than those of MWE cells (Fig. 9) and MWE (Fig. 8). Response latencies varied depending on the position of a whisker in the RF, as illustrated for a SWE cell (Fig. 9A) and a MWE cell (Fig. 9B). Excitatory responses had shorter latencies than inhibitory responses (Fig. 9C) and the variation of latencies of excitatory responses according to RF position was less pronounced than the variation of inhibitory responses. The EPSP in SWE and MWE cells had almost identical latencies, but the latency of the first AP was significantly longer in MWE cells (Fig. 9C and D; unpaired t test, P < 0.001). Stimulus onset responses of SWE neurones had remarkably small jitter (< 1 ms) and bursts of APs were infrequent (Figs 8 and 10). Responses to the stimulus onset of MWE cells exhibited a larger temporal dispersion (Figs 8A and B, and 10A and B), both when measuring only the first APs (unpaired t test, P < 0.01) and when measuring 90% of all APs (Fig. 10C; unpaired t test, P < 0.01). Finally, bursts of APs were more common in MWE cells (unpaired t test, P < 0.001).

**Deflection amplitude.** In a subset of cells (n = 12, all classified as either SWE or MWE neurones) the responses to stimuli of constant velocity but varying amplitude were studied. In the cell illustrated in Fig. 11A, a reduction of the PW deflection decreased the response to become subthreshold. Upon further reduction, the EPSP failed, leaving an IPSP as the most sensitive response component. The SuW responses had higher activation thresholds than PW responses (see also Fig. 11B). The responses of the two cells shown in Fig. 11A and B were characteristic for all cells studied (Fig. 11C). A decrease in amplitude also caused a substantial increase in response latency, particularly for excitatory responses. With small deflections, the IPSP could even precede the EPSP, which was never observed at high-amplitude (PW) stimuli (data not shown). No significant differences were found in the response thresholds of SWE and MWE cells.

The responses to stimuli of four different directions were studied in a subset of cells (n = 14). The excitatory and inhibitory response components clearly differed in their degree of directional preference (Figs 12A and B). In the cell shown in Fig. 12A, the EPSP amplitude changed about three-fold as a function of stimulus direction.
whereas the IPSP amplitude varied less than 1.5-fold. Suprathreshold responses had the clearest directionality (Fig. 12B). Similar observations were made on the population level (Fig. 12C). The directionality of SWE and MWE cells did not differ significantly. For subthreshold EPSPs and suprathreshold EPSP-APs, the overall distribution of preferred directions was strongly biased towards upward and backward whisker deflections. IPSPs, on the other hand, showed no directional preference (Fig. 12D).

**Intrinsic cell responses**

All cells, independent of their RF type, had resting membrane potentials around $-65\, \text{mV}$ (Table 2). Both inward and outward rectification was observed. However, the effective input resistance of cells of the different RF types differed, as MWE cells had significantly lower input resistances than SWE cells (ANOVA, $P < 0.01$) and other VPM cells. A weak negative correlation between the input resistance of the cell and its total dendritic length ($R^2 = 0.35$) was found. It should be mentioned that the inward and outward rectifying conductances of VPM cells are likely to affect the measurement input resistances.

**Active electrical properties.** Depolarisation by constant current injection produced regenerative potentials in all cells (Fig. 13A). In some cells, hyperpolarisation was also followed by a regenerative depolarisation that was

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**Figure 8. Membrane potential changes and AP responses for different RF types**

*Figure 8.* Membrane potential changes and AP responses for different RF types

A, SWE cell responses. Top: population average of potential changes to onset (left column) and offset (right column) of PW deflections. $P_{\text{ON}}, P_{\text{OFF}} =$ stimulus artefacts. Middle: peristimulus time histograms (PSTHs) of individual cells. Bottom: population PSTH. Bin width in the PSTHs is 0.5 ms. B, MWE cell responses. Same conventions as in A. Stimulus artefacts are partially blanked.
sometimes associated with a burst of APs. The regenerative potentials resembled, in their time course, low-threshold Ca\(^{2+}\) potentials, as described in *in vitro* preparations (Jahnsen & Llinás, 1984; for review see Steriade *et al.* 1997).

A number of observations indicated that these putative low-threshold Ca\(^{2+}\) potentials were also triggered by sensory stimulation. As illustrated in Fig. 13B, this was particularly common for the responses of MWE cells. The amplitudes of both stimulus onset and offset responses had a bimodal distribution (compare the upper and lower panels of Fig. 13B), caused by the triggering of an all-or-none regenerative event. The time course of this regenerative potential resembled that of the putative low-threshold Ca\(^{2+}\) potential evoked by current injection.

**Figure 9. Response latency**

*A*, response latencies at different RF positions of a SWE cell. Records with median latencies were selected for each stimulus position. The latency of the inhibitory response was 9 ms for SuW D1 and 20 ms for SuW D4. The excitatory response and the AP are clipped. *B*, response latencies at different RF positions of a MWE cell; conventions as in *A*. The latency of the excitatory response was 6.5 ms for D2 (PW) and 12.5 ms for SuW, responses. *C*, average response latencies for PW stimulation (upper) and SuW stimulation (bottom). *D*, plot of the modal EPSP latency against modal AP latency for PW deflection. EPSP latency is the time to 5% of the peak amplitude of the subthreshold response. AP latency is the time to AP peak. Stimulus artefacts are partially blanked.
In the suprathreshold cases, a slowly rising depolarising potential appeared to be triggered by the EPSP. The voltage threshold of the depolarising potential was roughly similar to the activation threshold of the current-evoked low-threshold Ca\(^{2+}\) potential. In those responses in which a large-amplitude Ca\(^{2+}\) potential was triggered during the onset response, a Ca\(^{2+}\) potential appeared to be less likely during the stimulus offset response (compare upper and lower panel in Fig. 13B). This finding is consistent with a slow removal of the inactivation of the low-threshold Ca\(^{2+}\) potential (Huguenard & McCormick, 1992). In other cells, current injections applied during sensory stimulation revealed that the occurrence of these large-amplitude responses evoked by sensory stimulation was dependent upon the membrane potential (data not shown), consistent with the activation and inactivation voltages of T-type Ca\(^{2+}\) channels (Llinás, 1988; Steriade et al. 1997).

Our data suggest that the generation of low-threshold Ca\(^{2+}\) potentials in the sensory responses of VPM cells was controlled by sensory evoked inhibition. Putative low-threshold Ca\(^{2+}\) potentials were more common in the sensory responses of MWE cells than in responses of SWE cells. Due to low-threshold Ca\(^{2+}\) potentials, MWE cells showed a broader and more prolonged depolarisation in the population response waveform than did SWE cells (Fig. 8). Moreover, low-threshold Ca\(^{2+}\) potentials contributed to the larger number of AP bursts observed in MWE cell responses than in SWE cell responses (Fig. 10C). Surprisingly, both SWE and MWE cells had the same tendency to discharge

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**Figure 10. Temporal scatter of SWE and MWE cell responses**

A, ten superimposed responses to PW deflection in a SWE cell (upper panel) and a MWE cell (lower panel). Stimulus artefacts are partially blanked. B, ordered plot of the temporal dispersion of SWE and MWE AP responses. C, occurrence of AP bursts (several AP within 10 ms) in SWE and MWE cell responses.
low-threshold Ca\(^{2+}\) potentials in response to current injection. We therefore argue that the absence of low-threshold Ca\(^{2+}\) potentials in the sensory response of SWE cells is likely to be due the strong action of sensory evoked inhibition in these cells (see the prominent population IPSP in SWE cells in Fig. 8).

**Anaesthesia**

The effects of urethane anesthesia in 3-week-old animals appeared to be similar in overall time course and depth to the effects of urethane anaesthesia as they have been described for adult animals (Friedberg et al. 1999; see also Armstrong-James (1975) for a description of the effects of urethane anaesthesia in very young (postnatal day, P, 7) animals). The RF size of VPM neurones increases substantially with decreasing depth of anaesthesia (Friedberg et al. 1999), raising the question as to whether the smaller RF size of SWE cells simply resulted from greater depth of anaesthesia in the respective recording conditions or from genuine differences between different cell types. Several observations make it unlikely that the differences between SWE and MWE cells could be accounted for by differences in anaesthesia:

1. During two SWE cell recordings it was necessary to supplement anaesthetic because the animal displayed slight whisker movements and strong withdrawal reflexes. Thus, these recordings showed that SWE responses can be observed in states of light anaesthesia (i.e. probably during anaesthetic state III-2; Friedberg et al. 1999).

![Figure 11. Responses to different stimulus amplitudes](image)

**A.** SWE cell responses to stimuli with different amplitudes for PW deflection (left column) and SuW deflection (right column). Records with modal response amplitudes are selected for each stimulus amplitude. In the uppermost trace on the left, the AP has been clipped. **B.** RF plots for the standard stimulus amplitude (6°, left) and a very small stimulus amplitude (0.06°, right). **C.** Distribution of various response components for different stimulus amplitudes for PW deflections (left) and SuW deflections. Stimulus artefacts are partially blanked.
We recorded from several cells from one animal, and in two cases we recorded SWE RFs 1.5 and 2.5 h after having recorded MWE RFs. Here, an additional dose of anaesthetic agent, anaesthesia was not supplemented between the two recordings. Since the depth generally decreases in the time between doses of anaesthetic agent, it seems unlikely that in these two cases a change from a MWE RF to a SWE RF was associated with a deeper level of anaesthesia.

The anaesthetic state in our experiments varied across recording time, but we did not observe a change in RFs from a SWE to a MWE response type when recording for up to 2.5 h from individual cells. While we did not observe changes in RFs from the SWE to the MWE type depending upon the depth of anaesthesia, it was obvious that the depth of anaesthesia affected the response properties. Light anaesthesia was associated with increased subthreshold background activity and stronger whisker-evoked responses. It seems likely that the size of suprathreshold RFs of MWE cells increases with lighter anaesthesia, compatible with the results of Friedberg et al. (1999).

DISCUSSION

We made in vivo whole-cell voltage recordings to elucidate how VPM neurones transform APs from different whisker afferents into the sub- and suprathreshold membrane potential changes that ‘represent’ whisker deflections in the thalamus. The results indicate that several classes of neurones exist that have widely different subthreshold RFs and different intrinsic membrane properties. Most neurones respond with initial EPSPs to PW deflection and the delayed IPSPs sharpen the differences in the spatial and

Figure 12. Responses to different stimulus directions

A, subthreshold responses to varying stimulus directions. $F_{\text{on}}$ = stimulator artefact. B, direction tuning of various response components, same cell as in A. C, directionality indices for various response components for a population of SWE and MWE cells. Values of 1 represent completely directional responses, values of 0 represent completely non-directional responses. Error bars represent 1 s.d. D, distribution of preferred directions for various response components. Stimulus artefacts are partially blanked.

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temporal characteristics of the two major functional cell types that convey the VPM output to the cortex. Since inhibitory inputs to the VPM are thought to arise from the thalamic reticular nucleus, the sharpening VPM output seems to arise from intrathalamic circuits. We did not find clear morphological differences in the dendritic arbors between the different functional cell types.

The results are mainly from young animals (P16–24), whereas most previous studies on VPM neurones report data from adult animals. Developmental studies on the mouse VPM indicate that major developmental changes of the dendritic architecture and the intrinsic as well as synaptic properties of VPM neurones occur before P16, although there are changes in dendritic architecture and intrinsic properties after P16 (Zantua et al. 1996; Warren & Jones, 1997). Some of our results are therefore likely to be particular for the young age of our animals. In particular, the lack of dendritic elaboration in some of the cells we recovered (Fig. 6A and B) may reflect the immature state of these cells.

**Figure 13. Low-threshold Ca\(^{2+}\) potentials**

Left column: A, current injection resulted as in almost all VPM cells in a regenerative depolarising potential. The regenerative potential resembled in its time course the low-threshold Ca\(^{2+}\) potentials (Steriade et al. 1997). B, ten responses to PW deflection recorded in the same MWE cell shown in A. Top: records in which depolarising regenerative potentials were triggered during the ON-response. Bottom: records, in which ON-responses remained subthreshold. APs have been clipped in A and B. Right column: depolarising potential triggered in the different conditions at higher temporal resolution.

**Spatiotemporal organisation of subthreshold RFs**

A salient property of VPM neurone responses in whole-cell voltage recordings was their antagonistic subthreshold RF organisation. For medium to large deflections, the majority of VPM neurones had RFs that were divided into excitatory and inhibitory whisker responses, as inferred previously from extracellular unit recordings in the thalamus of the visual system (Hubel & Wiesel, 1961; Sillito & Kemp, 1983). VPM neurones responded less vigorously to multi-whisker deflections than to movement of a single whisker, which might be one functional consequence of this antagonistic RF structure. Barrel cortex neurones, which do not show antagonistic RFs, respond equally well or even more vigorously to a multi-whisker stimulus compared to single-whisker deflection (unpublished data). In extracellular unit recordings, the low rate of spontaneously occurring APs in VPM neurones might have obscured the detection of these inhibitory RF zones, although indirect evidence for surround inhibition in the VPM exists (Simons & Carvell, 1989; Lee et al. 1994a).
Responses of SWE neurones were characterised by an initial EPSP that was terminated by a prominent IPSP, associated with an AP that was precisely locked to stimulus onset. In contrast, MWE cells had smaller IPSPs, and the associated APs were less precisely locked. These observations suggest that the IPSPs generated by reciprocal reticulothalamic connections delineate the pattern of suprathreshold VPM responses, consistent with the results obtained by pharmacological block or by lesion techniques (Lee et al. 1994 a,b; Hartings & Simons, 2000).

Previous studies have employed different types of anaesthesia as well as different mapping techniques to determine RF size, and report suprathreshold RF structures ranging from a strong predominance of single-whisker RFs (Waite, 1973a; Shosaku, 1985; Sumitomo & Iwama, 1987) to a predominance of multi-whisker RFs (Simons & Carvell, 1989; Nicolelis et al. 1993). Compared with studies using urethane anaesthesia, the predominance of single-whisker suprathreshold RFs reported here agrees with some (Waite, 1973a; Shosaku, 1985; Rhoades et al. 1987; Sumitomo & Iwama, 1987) but not with other reports (Armstrong-James & Callahan, 1991; Friedberg et al. 1999; Lee et al. 1994a,b). Large suprathreshold RFs were measured under anaesthetics other than urethane (Simons & Carvell, 1989; Nicolelis et al. 1993). Thus the depth as well as the type of anaesthetic seems to affect suprathreshold RF structure. We assume that the urethane anesthesia profoundly affected the VPM cells, because the activity of thalamic neurones is regulated as a function of states of sleep or wakefulness (see Steriade et al. 1997 for review).

The spatiotemporal response profiles of VPM neurones did not simply scale with stimulus amplitude. The subthreshold RF organisation of SWE and MWE cells changed from antagonistic multi-whisker RFs at large and intermediate deflections to primarily single-whisker RFs with responses at small deflections. These results might indicate that the contrast enhancement generated by thalamic lateral inhibition is not advantageous at the low signal-to-noise ratios when whisker deflections are small. The finding that IPSPs are more readily evoked by small-amplitude stimulation than EPSPs is, however, difficult to reconcile with the view that inhibition results exclusively from feedback via reticular interneurones. We speculate that a sparse population of VPM cells, not sampled in the present experiments, can activate interneurones of the reticular nucleus.

**Stimulus representation by subthreshold postsynaptic potentials and APs**

Mapping of responses to single-whisker stimuli revealed significant differences between subthreshold and suprathreshold RFs. While AP responses were restricted to a single whisker and consisted of one or a few APs within a narrow time window, the subthreshold responses (EPSPs and IPSPs) could always be elicited from several adjacent whiskers and lasted for hundreds of milliseconds. Such large differences between sub- and suprathreshold RFs have also been observed in cortical neurones (Bringuier et al. 1999; Moore & Nelson, 1999; Zhu & Connors, 1999). The contribution of subthreshold depolarisations to the neuronal representation of whisker stimuli remains to be explored. Presumably subthreshold depolarisations modulate the AP output of VPM cells during more complex whisker stimulation. Multi-whisker deflections induced by ‘whisking’, are presumably complex patterns of stimuli varying in number of whisker and time. How such complex stimuli are represented electrically in the thalamus cannot be understood without knowing the structure of subthreshold RFs.

**Subthreshold RFs and dendritic morphology**

The spatial and temporal response characteristics and passive membrane properties of VPM neurones did not vary independently across our sample, but were clustered. SWE cells differed from MWE cells in the following properties: (a) they had a smaller (single-whisker) excitatory subthreshold RF, (b) when APs were elicited they had shorter latencies, (c) they showed less variability of AP number per PW deflection within and across cells, (d) they had less temporal scatter in their AP responses, (e) they received stronger inhibition, (f) fewer AP-bursts were evoked as well as putative low-threshold Ca2+-APs, and (g) they had higher effective input resistances.

While several studies have suggested the existence of subclasses of somatosensory neurones in the cat thalamus (Yen et al. 1985; Turner et al. 1997), neurones in the rat VPM have been viewed as a homogeneous population (Harris, 1986). The segregation of SWE and MWE cells is different from the distinction of X- and Y-cells in the lateral geniculate nucleus of the visual system. In the visual thalamus, the temporally more precise Y-cells have larger RFs than X-cells (Cleland et al. 1971), opposite to the situation encountered with SWE and MWE cells in the somatosensory thalamus.

The question of whether differences in cell response properties (SWE or MWE) are related to morphological differences remains, as yet, unresolved. Our sample size was small and the polymorphism within the RF type of reconstructed cells seems to indicate a lack of correlation. This polymorphism of dendritic arbors, and the lack of dendritic elaboration in some of the SWE cells (Fig. 6A and B) might be related to the young age of the animals (with incomplete dendritic maturation). The differences in input resistance between SWE and MWE cells might, however, indicate morphological differences. Alternatively, SWE and MWE cells might differ in their specific membrane resistances, as suggested for X- and Y-cells in the visual thalamus (Bloomfield et al. 1987; Crunelli et al. 1987).
The neurones described here compare in morphology to those described previously in the rodent VPM (Harris, 1986; Chiaia et al. 1991; Ohara & Havton, 1994; Zanuta et al. 1996) and other thalamic nuclei (for review see Steriade et al. 1997). A relationship between RF size and dendritic arbor extension in VPM neurones was not established (Chiaia et al. 1991).

Functional properties might, however, be linked to characteristics of dendritic morphology such as the elongation of dendritic field along barrelloid rows and arcs, and was related to the elongation of RFs along whisker rows and arcs. The dendritic morphology of VPM neurones is very different from that of their major target neurones, the spiny stellate cells in cortical layer IV. These cells have smaller somata, and substantially longer dendrites, which are confined to barrel borders (Woolsey et al. 1975; Simons & Woolsey, 1984; Lübke et al. 2000; Petersen & Sakmann 2000), unlike the VPM dendritic arbors, which do not seem to be confined to barrelloid borders. It will be important to determine whether SWE and MWE cells differ in their axonal projections to the cortex, especially since a recent intracellular labelling study (Arnold et al. 2001) has described two distinct types of thalamocortical axon arborisation. It would be most interesting to find out whether SWE cells have single barrel of projecting axons and MWE cells have bifurcating-type axons, as has been described by Arnold et al. (2001).

### Intrinsic mechanisms contributing to RF structure

The temporal precision of the EPSP-AP responses of SWE cells is achieved by a number of cellular mechanisms. Inward- and outward-rectifying conductances appear to stabilise the resting membrane potential at values close to the AP threshold, EPSPs were large and had a fast rise time, the high input resistance of SWE cells presumably sped up depolarisation, and a large and exactly timed IPSP terminated the response and prevented ongoing afferent input as well as low-threshold Ca$^{2+}$ potentials from evoking additional APs.

The weak direction tuning of evoked IPSPs suggests that the directionality of VPM responses is not primarily dependent upon inhibition, consistent with the results from pharmacological block of inhibition (Lee et al. 1994b) and RF properties of the inhibitory reticular neurones (Shosaku, 1985).

Low-threshold regenerative Ca$^{2+}$ potentials could amplify sensory inputs (Williams & Stuart, 2000). The occurrence of low-threshold Ca$^{2+}$ potentials is thought to be controlled by wakefulness/sleep-associated steady-state membrane potential fluctuations (Steriade et al. 1997 for review). A contribution of low-threshold Ca$^{2+}$ potentials to sensory transmission in the VPM has been suggested previously (Salt & Eaton, 1990). The fact that the resting membrane potential of VPM cells was just below the inactivation voltage of T-type Ca$^{2+}$ channels might contribute to the complexity of the interaction of sensory inputs and low-threshold Ca$^{2+}$ potentials (Steriade et al. 1997).

### Functional implications

The present results raise the question of how the different classes of neurones in the VPM with different RFs contribute to electrical representation of whisker movement in the cortex, as measured by extracellular unit recording (Armstrong-James, 1995; Simons, 1995) or by voltage-sensitive dye signals (Kleinfeld & Delaney, 1996) or intrinsic fluorescence signals (Polley et al. 1999). In SWE neurones, the time window between the initial EPSP and the AP triggered by a PW deflection is less than a millisecond and their output is not graded. In MWE cells, the situation is the opposite as their responses were more graded, varying between subthreshold EPSP responses to suprathreshold EPSPs evoking several APs. While MWE cells might encode a stimulus property like the degree of deflection by the number of APs, the single AP responses of SWE cells might encode solely the exact time of occurrence and location of the whisker deflection.

Temporal stimulus cues may be encoded by the electrical activity of the rat somatosensory system during the fast exploratory ‘whisking’ movements. At realistic whisking speeds of 1500° s$^{-1}$, two whiskers of an arc might collide with an upright obstacle simultaneously, whereas a 10° tilted object will result in a roughly 1 ms delay between the two collisions (Welker, 1964, Carvell & Simons, 1990). SWE cells in the VPM have the relay characteristics required for recovering spatial information about objects from such temporal sequences of whisker deflections via a sub-millisecond time resolution at the single-whisker level. Therefore, the two major cell types of VPM neurones are likely to convey stimulus cues either primarily by timing of APs (SWE cells) or by the number of APs (MWE cells).

### REFERENCES


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