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RESEARCH ARTICLE

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Balancing bilateral sensory activity: callosal processing modulates sensory transmission through the contralateral thalamus by altering the response threshold

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Abstract Rats tactually explore a nearly spherical space field around their heads with their whiskers. The information sampled by the two sets of whiskers is integrated bilaterally at the cortical level in an activity dependent manner via the corpus callosum. We have recently shown that sensory activity in one barrel field cortex (BFC) modulates the processing of incoming sensory information to the other BFC. Whether interhemispheric integration is dynamically linked with corticothalamic modulation of incoming sensory activity is an important hypothesis to test, since subcortical relay neurons are directly modulated by cortical neurons through top-down processes. In the present study, we compared the direct sensory responses of single thalamic relay neurons under urethane anesthesia before and after inactivating the BFC contralateral to a thalamic neuron. The data show that silencing one BFC reduces response magnitude in contralateral thalamic relay neurons, significantly and reversibly, in response to test stimuli applied to the principal whisker at two times response threshold (2T) intensity for each unit. Neurons in the ventral posterior medial (VPM) nucleus and the medial division of the posterior nucleus (POm) react in a similar manner, although POm neurons are more profoundly depressed by inactivation of the contralateral BFC than VPM neurons. The results support the novel idea that the subcortical relay of sensory information to one hemisphere is strongly modulated by activity levels in the contralateral as well as in the ipsilateral SI cortex. The mechanism of the modulation appears to be based on shifting the stimulus–response curves of thalamic neurons, thereby rendering them more or less sensitive to sensory stimuli. We conclude that global sensory processing is created by combining activity in each cerebral hemisphere and continually balancing the flow of information to cortex by adjusting the responsiveness of ascending sensory pathways.

Keywords Barrel cortex · Somatic sensory processing · Perception · Muscimol · Corpus callosum · Rat · Whiskers

Introduction

Rats use their whiskers to explore the space around their heads in a manner analogous to humans using their hands to negotiate space in the dark. Sensory information from the roughly 25 whiskers on each side of a rat's face is projected to the contralateral cortex through pathways that are thought to dynamically update the representation of objects in space, in most cases within the touching distance of the moving whiskers. The pathway from sensory receptors in whisker follicles to the contralateral cortex consists of "barrelettes", clusters of neurons in the ipsilateral brainstem trigeminal complex (Ma 1991), which project to "barreloids" in the contralateral thalamus (Van der Loos 1976), and "barrels" in the primary somatosensory (SI) cortical layer IV; with one barrel for each whisker (Woolsey and Van der Loos 1970). This lemniscal component of the whisker to barrel ascending pathway in rats is highly lateralized subcortically (Peschanski 1984; Chiaia et al. 1991). Accordingly, objects on the left side of a rat's face are represented initially by neurons in the right barrel cortex, and vice versa.

Previous studies have shown that barrel neurons respond most strongly to stimuli applied to the contralateral whiskers, but evidence also supports that cortical neurons are influenced by inputs from the whiskers on the ipsilateral side of the face (Harris and Diamond 2000; Pidoux and Verley 1979; Shuler et al. 2001; Wiest et al. 2005). The responses of cortical cells to ipsilateral

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whiskers are generated by interhemispheric connections through the corpus callosum, based on the observation that blocking activity in one hemisphere eliminates bilateral responses in the other hemisphere (Pidoux and Verley 1979; Shuler et al. 2001). The behavioral relevance of integrating information from the two sides of the face has been demonstrated by learning problems that require the animal to compare whether movable walls on the left and right side are close to or far from the head using only their whiskers (Krupa et al. 2001; Shuler et al. 2002).

Thus, bilateral integration requires cortex to interact with the levels/types of sensory information coming in from both sides of the face. To detect disturbances in left and right side input activity, cortex in each hemisphere has to compare activity levels in the sensory pathways that bring sensory information into left and right sides of the system, even at subcortical levels. Anatomically, projections from barrel field cortex (BFC) back to thalamus greatly outnumber the ascending projections from brainstem to thalamic relay neurons (Guillery 1969; Liu et al. 1995), which correlates with the functional evidence that the thalamic "relay" cells are strongly modulated by cortical feedback (Diamond et al. 1992b; Nicolelis et al. 1995). Corticothalamic projections from layer VI in one barrel column of BFC have a general facilitatory effect on the relay cells in the corresponding barreloid (Temereanca and Simons 2004). Moreover functional reorganization, in the form of shifts in the spatiotemporal structure of the receptive fields (RFs) in ventral posterior medial (VPM) nucleus (Krupa et al. 1999), had been reported after BFC neuronal activity was reversibly blocked. Recent studies have demonstrated direct facilitatory effects of sensory cortex on thalamic "relay" nuclei via corticothalamic "feedback" projections (for review, see Sherman and Guillery 2002; Suga et al. 2003).

Several lines of evidence have emerged showing that activity levels in one hemisphere can regulate sensory response levels in the other hemisphere. Thus, the interhemispheric integration may interact with corticothalamic coordination. For example, decreases in neuronal activity in one barrel cortex, caused by either a lesion (Rema and Ebner 2003) or by chronic suppression of activity (Li et al. 2005), produce a remarkable decrease, even in the early 3–10 ms response component, which is the earliest response component that is often assigned to activation of cortical neurons by thalamic inputs (Armstrong-James and Fox 1987; Armstrong-James et al. 1991). These findings, taken together, led us to test the hypothesis that cortex in one hemisphere can indirectly modulate the responsiveness of relay neurons in the contralateral thalamus.

In the present study, response curves of thalamic relay neurons were measured by systematically varying the amplitude of test stimuli under urethane anesthesia. Responses of the same thalamic relay neurons to whisker stimuli were compared before and after inactivating the contralateral BFC using stimulus intensities of only twice the response threshold (2T). Under these conditions (urethane anesthesia, tested at 2T), the responsiveness of VPM and the medial division of the posterior nucleus (POm) neurons to principal whisker stimuli is significantly depressed after inactivating the contralateral BFC with muscimol in the absence of any CNS damage. Our data also show that response curves of thalamic relay neurons are greatly shifted toward the right after the suppression of neural activity in the contralateral BFC. Thus, silencing cortex down-regulates responses to sensory inputs in the thalamus bilaterally. Our findings suggest that cortex in one hemisphere can indirectly modulate the responsiveness of relay neurons in the contralateral thalamus, most likely by down-regulating excitability in the contralateral cortex.

Materials and methods

Sixteen male Long-Evans rats (250–350 g or 2–3 months old) were used for these experiments. Changes of spontaneous activity (SA) and evoked responses (ER) to repetitive whisker stimulation before and after inactivation of contralateral BFC by muscimol were systematically investigated in eight animals (one neuron per animal). The influence of contralateral cortical inactivation on POm was studied in four other rats. Controls consisted of VPM neurons that were monitored before and after muscimol was applied *only* onto the surface of the contralateral **visual** cortex using the same paradigm as in our experimental group ($n = 4$ rats). The experimental design is illustrated in Fig. 1. All procedures were approved by the Vanderbilt University Institutional Animal Use Committee and followed guidelines by National Institutes of Health and Society of Neuroscience.

Chamber implantation

Detailed description of the surgery, electrophysiology, and histology has been published elsewhere (Li et al. 2005; Rema and Ebner 2003) and will be briefly repeated here. Urethane anesthetized rats (1.5 g/kg, 30% aqueous solution, i.p.) were placed in a stereotaxic headholder (Narashige). Body temperature was maintained at 37°C by rectal thermometer feedback to an electronically controlled heating pad. In 12 experimental animals, a craniotomy was performed in the right skull to make a circular opening with a fine dental burr. The opening was centered at 2 mm posterior and 6 mm lateral to Bregma with ~4 mm in diameter, which provided access to the right BFC. Care was taken to avoid any injury to dural or cortical blood vessels, and gel foam was applied in case of bleeding. After the surface of the bone surrounding the opening was dried with gauze, super glue was carefully applied onto the bone surface around the opening. A small plastic chamber was placed above the opening then cemented watertight to the skull with

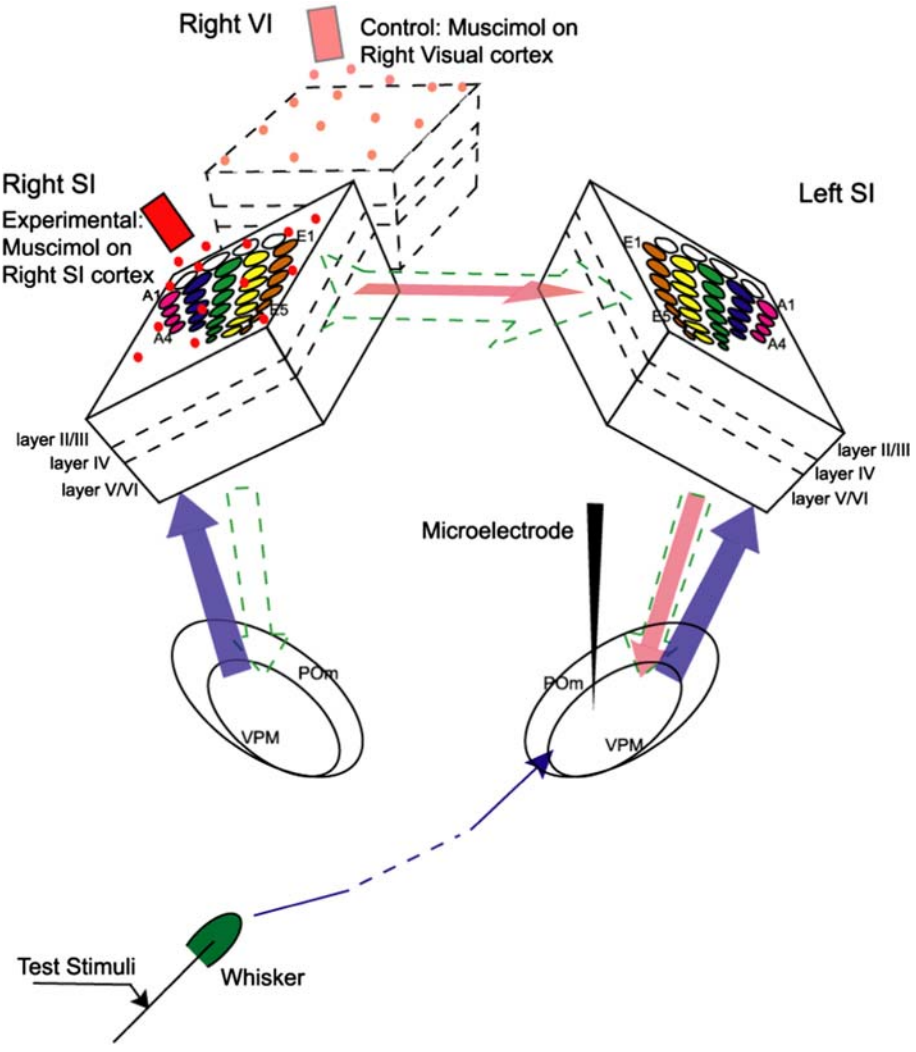

Fig. 1 Experiment design. Illustration of the experimental design and working hypothesis. BFC neurons on both sides are interlinked in an activity dependent manner in normal rats. When the right SI is inactivated, influence from the blocked BFC would decrease significantly (*horizontal red arrow*). We postulate that this could down-regulate the influence of the corticothalamic projections (*red arrow*) from unmanipulated BFC (*left*). Hence the neurons in the left VPM could reduce their responsiveness to peripheral whisker stimulation

dental cement. The exposed cortical surface within the chamber was covered with warm saline to prevent drying. Another rectangular opening was made in the left skull to gain access to the left (contralateral) thalamus. This opening exposed the cortical surface 2–5 mm posterior and 2–4 mm lateral to Bregma. The bone debris was carefully removed with saline during drilling.

In the control group, a circular opening was made on the right skull to expose the right visual cortex instead of the right SI cortex. The center of the opening was located at 6 mm posterior and 2 mm lateral to Bregma (Li et al. 2005). Similar to the experimental group, a small opening was made in the dura with a 32-gauge syringe needle and the chamber was cemented over the opening. The rectangular opening to gain access to the thalamus was made at the same location as in the experimental group.

Electrophysiological recording

Extracellular single unit recording was conducted with carbon-fiber microelectrodes (Armstrong-James and Millar 1979; Armstrong-James et al. 1980). The microelectrode was advanced into the thalamus vertically through small slits in the dura by a stepping motor microdrive (Kopf instruments). Contact of the electrode tip with the pial surface was identified visually under an operation microscope and by reduction in noise when the electrode touched the brain surface. Single units were isolated on-line with a time–amplitude window discriminator (Bak Instruments). Spikes were digitized with the CED 1401 Plus processor (Cambridge Electronic Design) and peri-stimulus time histograms (PSTHs) were built on-line to visualize the response characteristics of each isolated unit with bin width of 1 ms using Spike 2 software (Cambridge Electronic Design). The unit candidate had to be clearly distinguished by its waveform from other units around. To ensure the quality of the single unit isolation, the unit with the largest waveform was typically selected at each penetration depth. Neuronal activity of both ER and SA was recorded for more than 1 h before muscimol application as a criterion for satisfactory stability. Spike duration of all VB units isolated was recorded in a notebook for later reference. Data collected before muscimol appli-

cation established the baseline for each cell. During baseline recording, supplements of anesthetic (10% of initial dose) were given as needed to maintain the anesthesia level at stage III-3 (Friedberg et al. 1999). At this anesthetic depth, VPM units display low spontaneous firing (Waite 1973b) and respond to 1 Hz whisker stimulation without inducing bursts (Diamond et al. 1992a; Friedberg et al. 1999; Sherman and Guillery 1996). Data was stored on a PC (Dell) for further off-line analysis.

Receptive field mapping

The location of VPM or POm was estimated by X–Y coordinates linked to the depth read-out of the microdrive (Brown and Waite 1974; Haidarliu and Ahissar 2001; Ito 1988; Land et al. 1995; Sugitani et al. 1990; Waite 1973a, b). When the electrode was in the dorsal thalamus, whiskers were deflected by a hand-held probe to test for responses. When the microelectrode was judged to be in VPM and a single unit with a good response to manual whisker deflections was isolated, all whiskers on the right side of the face were trimmed to a length of 10 mm. Under urethane anesthesia stage III-3, neurons in dorsal VPM exhibited small RF size (1–2 whiskers) with low spontaneous firing (Diamond et al. 1992a; Friedberg et al. 1999; Waite 1973b; but see Rhoades et al. 1987; Timofeeva et al. 2003). Only units with C or D row whiskers as their principal whisker were selected to go through the silencing and recovery procedure since these barreloids localized in the middle of VPM which facilitated our selection of VPM units. The RFs of VB neurons were initially mapped with a hand-held probe to identify the principal whisker. A bimorph piezoelectric stimulator was used to refine the RF with 50 ramp-and-hold forward deflections to each whisker delivered 10 mm away from the face at the rate of 1 stimulus per second. The ramp and hold stimulus was 300 μm in amplitude and 3 ms in duration. After the threshold (see below) was measured, the RF of this unit was re-mapped at 2T.

Threshold assessment and baseline recording

The piezoelectric wafer was driven by a digital stimulator (DS8000, WPI Inc.). We calibrated the piezoelectric wafer under a high-speed digital camera (Redlake Corp; 500 frames per second) to translate the voltage applied to the piezoelectric wafer into the distance in μms that the whisker was actually displaced. VPM neurons responded to whisker stimuli with higher firing probability and shorter modal latency than POm cells (Diamond et al. 1992a). For recording in VPM, if one isolated unit did not generate a robust response with a prominent response peak and modal response latency at ~5 ms to the test stimuli, it was bypassed and the electrode was advanced until another unit was located. When the isolated unit produced "good" responses, the response threshold to principal whisker stimulations was determined. Here threshold was defined as the lowest amplitude of whisker deflection (i.e., lowest voltage applied to the piezoelectric stimulator) that produced a statistically significant response (operationally, at least three spikes in one of the poststimulus bins in the PSTH, see the Method section of Armstrong-James and Fox 1987 for mathematical implications). The threshold was determined by varying the amplitude of whisker deflections in a linear-interpolation manner. For instance, if a 200 μm deflection evoked a robust response, the next test stimulus would be at 100 μm; if the 100 μm deflections generated no response, the next stimulation would be at 150 μm; and so on. When two successive test stimuli were close in amplitude (< 50 μm), the amplitude of test stimuli was increased in 10 μm steps to get an accuracy of ± 10 μm. After the threshold voltage was established, the responsiveness of this unit was recorded at a voltage of 2T. At each assessment time, a recording session consisted of three or four ER blocks, with one SA recording session randomly inserted. Each ER block documented the 3–100 ms poststimulus time neuronal activity generated from 50 stimuli delivered at 1 per second (bin width, 1 ms). The SA block recorded 200 s of spontaneous firing when the stimulator was still in contact with the whisker without activating the stimulator.

Muscimol application

Muscimol solution was prepared prior to each experiment by dissolving muscimol (Sigma) in sterile saline (10 mM). When the isolated unit showed stable responses, i.e., each ER block gave a similar number of spikes over a period of time longer than 1 h, warm saline within the cemented chamber was removed and the surface of exposed dura was cleaned. One small opening was made in the dura and a small piece of gelfoam saturated with the 10 mM muscimol solution was placed in the chamber to seamlessly cover the exposed dura. Responsiveness of the same isolated thalamic unit was tested periodically from the onset of muscimol application. The postmuscimol recording usually lasted about 1 h and drops of muscimol solution were added to the gelfoam to keep it moist. We also re-tested the threshold in some units after muscimol application. We continued to monitor the anesthesia depth after muscimol application as described in previous studies (Friedberg et al. 1999), and supplements (10% of the original dose) were given when necessary to maintain the anesthesia at stage III-3. In most of the cases, no signs of dramatic changes of anesthesia depth (i.e., corneal/hindlimb pinch reflex, voluntary whisker movements, etc.) were noticed in the 1-h post muscimol application recording. Only ER blocks collected when the isolated unit was in tonic firing mode were included in the data analysis. ER blocks when the unit responded in the bursting firing mode

were excluded, based on the interspike interval of poststimulus spikes.

Several criteria were used to select units: they had to (1) be clearly distinguished by their waveform from other units, (2) maintain their initial response to stimulation for at least 1 h, (3) have a stable modal response latency, (4) show a single whisker that produced the best response, (5) respond well at 2T stimulus levels, and (6) be confirmed histologically to be in the VPM or POm thalamic nucleus. We chose to study the response characteristics of each unit carefully and thoroughly before we applied muscimol and required each unit to meet "rigorous" criteria. Although these requirements greatly reduced our sample size, it enhanced the quality of our recording due to the fact that only stable neurons reliably detected effects of blocking contralateral BFC.

Data analysis

A PSTH was generated for each ER block with bin width of 1 ms in off-line data processing. Spikes collected prior to 3 ms poststimulus were rejected as being too early to be responses evoked by whisker stimulation. Spontaneous activity was corrected by subtracting the average number of spikes in the 50 ms prestimulus time bins from each poststimulus time bin. Spikes within 3–100 ms poststimulus time were summed for 50 stimuli to calculate the response magnitude (spikes/50 stimuli) and divided by 50 to get spikes/stimulus. Response magnitude was averaged from ER blocks at the same stimulus intensity and the same recording time point to calculate the baseline response. The average response magnitude was plotted against the stimulus intensity to construct the intensity–response curve for each unit. For data sampled at 2T, the average response magnitude at different time points during baseline recording was compared to identify unstable units. Latency histograms (LHs) were constructed from histograms that include only the first spike after each stimulus to assess onset latency and modal response latency. Data acquired after muscimol application were processed in the same manner. Spontaneous activity was analyzed separately.

Both the response magnitude and latency were compared before and after muscimol application to analyze the effect of silencing the contralateral BFC. For each unit, the average response magnitude at 2T was plotted against poststimulus time. Data from ER blocks at 2T in baseline recording were further pooled together and compared with data assessed at each recording time point after muscimol application. Student's t test was performed to identify significant differences between groups. Frequency distributions of the modal response latency were generated and compared between premuscimol and postmuscimol recording to determine whether there was any latency shift after muscimol. The intensity–response curves from the same unit were compared between baseline and postmuscimol application when possible.

To amalgamate several cases of each type for comparison, data from different VPM neurons were normalized. For each VPM unit, the normalization procedure took the averaged neuronal response at 2T in premuscimol recording as 100%, and data at every recording time point after muscimol application (3–4 recording blocks) were normalized by this value. Then the normalized values from different animals were grouped by recording time points, averaged and plotted against the time. This normalization procedure emphasized response *changes* within each VPM unit over time and between different VPM units at each time point so that data from different animals could be compared. Student's t test was performed on the data to determine significance levels.

Histology and identification of recording sites

On completion of recording, recording sites were marked by passing a DC current (electrode tip positive) of 2 μA for 10 s. After making a lesion, the rat was overdosed with urethane and perfused transcardially with PBS followed by 4% paraformaldehyde in buffer. Brains were saturated in 10, 20, and 30% sucrose, blocked and sectioned coronally, and stained for cytochrome oxidase activity (Wong-Riley and Welt 1980) to localize thalamic lesion sites.

Results

Histology confirmed the location of eight VPM (muscimol on contralateral BFC), four VPM (muscimol on contralateral visual cortex), and four POm (muscimol on contralateral BFC) units in 16 animals. Response characteristics of these units before muscimol application were similar to those reported for these nuclei in previous studies (Armstrong-James and Callahan 1991; Diamond et al. 1992a; Simons and Carvell 1989; Waite 1973b). Under our experiment design, only one neuron could be studied per animal due to the possibility that muscimol could produce long-term changes that could bias repeated results. There was no reliable way to control for possible contaminating effects of the muscimol application procedure. This factor, together with our unit-selecting criteria, resulted in the small sample size. However, as shown in the following text, each unit responded stably to test stimuli for the duration of our observation. More importantly, the highly consistent response changes among units (i.e., among different animals) after muscimol application supported the validity of the conclusions.

Response of thalamic relay neurons to whisker stimuli of various intensities

The data confirmed that VPM neurons are very sensitive to whisker deflections. The lowest response threshold

measured was ~20 μm in amplitude. For the 12 VPM neurons the threshold ranged from ~50 to 150 μm, with a mode of ~100 μm. When the stimulus was stronger (for example, >300 μm), VPM neurons fire ~50 spikes/50 stimuli or 1 spike/stimulus with a short modal latency (~5 ms). It was important that the cells be in the tonic firing mode, in which the VPM neurons are rapidly inhibited by feedback from the thalamic reticular nucleus (TRN) after activation by whisker stimulation (for review, see Castro-Alamancos 2004), since any changes in the firing mode would invalidate direct comparisons of response magnitude. As the stimulus intensity decreased, VPM neurons generated fewer spikes with longer response modal latency. At threshold, VPM neurons generated roughly <20 spikes/50 stimuli, and these spikes occurred with temporal dispersion in a PSTH. Increasing the stimulus by an additional 10 μm often identified the threshold because the cell transitions from a few scattered spikes/50 stimuli to a significant response to stimulation (Fig. 2a, b). That is, the VPM neurons' transition from nearly unresponsive to many poststimulus spikes aggregated in a narrow poststimulus time window. Generally speaking, at 2T, VPM neurons fired below asymptote, producing ~40 spikes/50 stimuli. Although the threshold varied from one VPM neuron to another, their stimulus intensity–response magnitude curves shared several common features. Figure 2a shows the intensity–response curves of two VPM neurons. All response curves were roughly sigmoid in shape and the slope after threshold was steep. The steep slope near threshold indicates dramatic changes of the responsiveness may occur during this "sensitive" epoch. Figure 2b shows the average PSTHs generated by different stimulus intensities in these two VPM neurons, respectively. For example, in VPM neuron 1, when the stimulus was just below threshold (100 μm in this case), only a few spikes were generated per 50 stimuli and they were scattered in the PSTH; at threshold (110 μm) the VPM neuron gave a response peak, while at 10 μm above threshold the spike number was substantially larger. At 2T, the response was very robust. Increasing stimulus intensity also shortened the response modal latency in all VPM neurons we recorded, as exemplified in Fig. 2b. These response characteristics were very consistent across all VPM neurons included in the present results.

Muscimol application influences both the response magnitude and latency of VPM neurons

Muscimol on the contralateral BFC did not alter the RF size, or affect the spontaneous firing of VPM units. However, within 30 min after muscimol was applied to the contralateral BFC, each thalamic neuron showed a significant decrease in whisker-ER magnitude, as well as a shift of the response modal latency to principal whisker stimulation (Fig. 3a, b). As shown in Fig. 3a, the influence of muscimol depended upon its application time. The time course of muscimol did vary slightly among VPM units, especially during the "initial phase". Shortly after muscimol was applied onto the contralateral BFC, the responsiveness of VPM neurons remained unchanged, or even increased in some units (Fig. 3a). However, after reaching a "turning point", the response magnitude of the VPM neurons began to drop.

Response reduction developed a similar temporal course in all eight VPM units. When normalized data from eight individual cases were averaged, the first significant change of VPM neuron responsiveness appeared at ~20 min after muscimol application (Fig. 3b). VPM neurons responded in the first 15 min at 102% ($P < 0.36$, t test) of the premuscimol magnitude level, but the response decreased to 92% ($P < 0.004$) at ~20 min then further reduced to 77% ($P < 6.4\times10^{-16}$), 69% ($P < 6.5\times10^{-20}$), 65% ($P < 1.2\times10^{-25}$), and 59% ($P < 2.5\times10^{-17}$) at 30, 40, 50, and 60 min after muscimol was applied, respectively. Another interesting feature is that VPM neurons elongate their modal response latency after muscimol. VPM neurons responded to whisker stimuli at 2T faithfully with very short modal response latency (~5–7 ms). However, at some time after muscimol was applied, VPM neurons began to respond to the same 2T test stimulus with longer modal response latency and reduced response magnitude. Details are highlighted by analyzing data from two of the eight VPM neurons (case VPM04 and VPM02). Before muscimol application, neuron VPM04 (Fig. 4f) was marked by small RF size (one whisker, Fig. 4c), short modal response latency (5 ms, Fig. 4a, top panel) and robust and stable response to its principal whisker D2 (Fig. 4b). However, unit VPM04 gradually but steadily changed its response to principal whisker stimulation after muscimol was applied: response magnitude was decreased and response temporal structure shifted. This response modification is clearly seen when PSTHs before and during muscimol application were averaged and compared individually for each case (Fig. 4a). Figure 4b shows that muscimol had no effect in the first 20 min: 21 min after muscimol was applied unit VPM04 generated an average of 40 spikes to 50 principal whisker stimuli, which is 81% ($P < 0.06$) of its normal value. This percentage was reduced to 73% ($P < 4\times10^{-4}$) at 29, 68% ($P < 0.02$) at 38, 57% ($P < 0.004$) at 47, and 52% ($P < 7\times10^{-4}$) at 56 min, respectively.

Inactivation of contralateral BFC by muscimol also changed the temporal structure of this unit's response to the peripheral stimulus. The change can be demonstrated by comparing two *averaged* PSTHs before and during muscimol application (Fig. 4a). Before muscimol application, modal response latency of unit VPM04 was 5 ms (13 of 13 recording blocks, Fig. 4d), and after muscimol was applied, only 77% (20 of 26 blocks) gave a modal latency still at 5 ms, while the modal response latency shifted to 8 ms in 23% (6 of 26) of recording blocks (Fig. 4d). This new 8 ms modal latency first appeared at 21 min after muscimol was applied, which correlates with the onset of the initial significant response reduction.

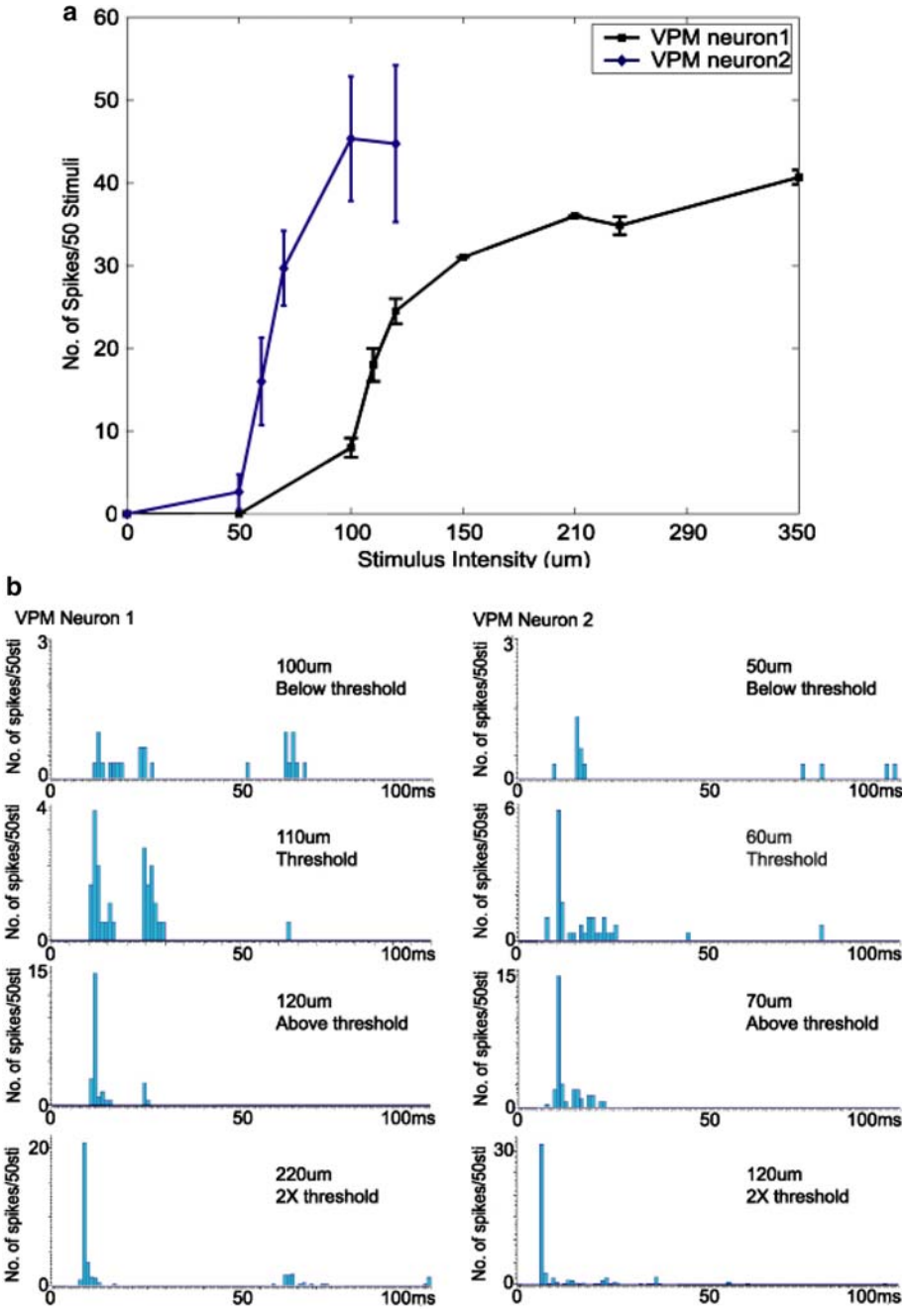

Fig. 2 Response curves of two selected VPM neurons. **a** Response curves of VPM neuron 1 (*black*) and VPM neuron 2 (*blue*) before muscimol application. Data were collected using standard whisker stimuli of various amplitudes (see Materials and Methods for details) at the rate of 1 stimulus per second. Response magnitude (spike count/50 stimuli) was plotted against the extent of whisker deflections (in μm). The threshold of these two VPM neurons is 110 and 60 μm under our criteria (see Methods). *Error bar*: SEM. **b** Averaged PSTHs (see Methods) for a narrow range of stimulus intensities. *Left column*: data from VPM neuron 1. From *top to bottom*: PSTH to 100 μm stimulus (8 ± 1 spikes/50 stimuli, mean ± SEM averaged from 150 trials); PSTH to 110 μm stimuli (18 ± 2 spikes/50 stimuli, averaged from 100 trials); PSTH to 120 μm stimuli (24.5 ± 1.5 spikes/50 stimuli, averaged from 100 trials); PSTH to 220 μm stimuli (33 ± 1 spikes/50 stimuli, averaged from 300 trials). *Right column*: data from VPM neuron 2. From *top to bottom*: PSTH to 50 μm stimuli (2.7 ± 2 spikes/50 stimuli, averaged from 150 trials); PSTH to 60 μm stimuli (16 ± 5 spikes/50 stimuli, averaged from 150 trials); PSTH to 70 μm stimuli (30 ± 4.5 spikes/50 stimuli, averaged from 150 trials); PSTH to 120 μm stimuli (45 ± 9 spikes/50 stimuli, averaged from 350 trials). Note that the latency systematically became shorter and shorter as the amplitude of whisker deflections increased (*y*-axis: spike count/50 stimuli; *x*-axis: 1 ms bins. Note change in *y*-axis scale)

The time-dependent influences of muscimol on the magnitude and temporal structure of responses in unit VPM04 were demonstrated in Fig. 5 by comparing the averaged PSTHs at different time points based on responses generated at 2T stimulus strength. Unit VPM04 originally responded to test stimuli with two peaks at 5

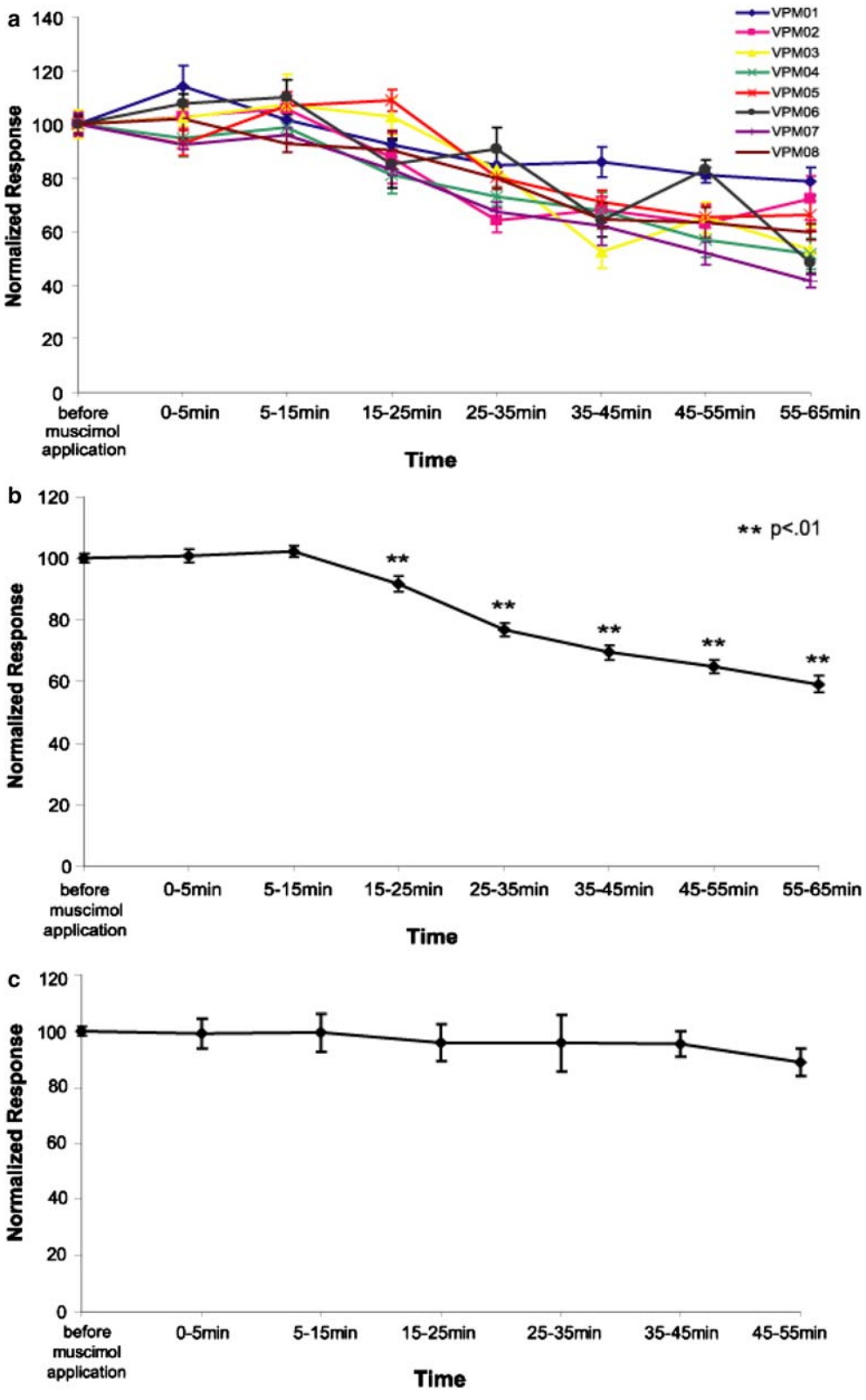

Fig. 3 Changes of response magnitude in eight VPM neurons after muscimol application. **a** Time course of normalized response magnitude in eight VPM neurons after muscimol application. Normalized response magnitude (taking the average control response level of each unit before muscimol as 100) was plotted against the postmuscimol time. Colors identify data from eight different individual VPM neurons (1 per animal). **b** Grand average response levels for the eight VPM neurons after normalization. Data from different animals were averaged over time. Inactivating the contralateral BFC had an overall suppressive influence on VPM neurons. *Asterisks* show that significant decreases first occur in the 15–25 min time window. **c** Response levels in control animals (muscimol on contralateral visual cortex). No decrease in response magnitude was detected in controls. Normalized data from four control animals were also plotted against the time. No significant decrease in response magnitude was found after muscimol application

and 8 ms, respectively (Fig. 5a). We continued to test the neuron repetitively for >2 h. The cell responded to test stimuli with almost identical PSTHs each time, for >2 h (Fig. 5b, c). Thus, we classified unit VPM04 as a reliable unit in a stable animal preparation. However, the response peak at 5 ms lowered significantly after the contralateral BFC was inactivated for 29 min, while the peak at 8 ms increased, and then decreased (Fig. 5d–f).

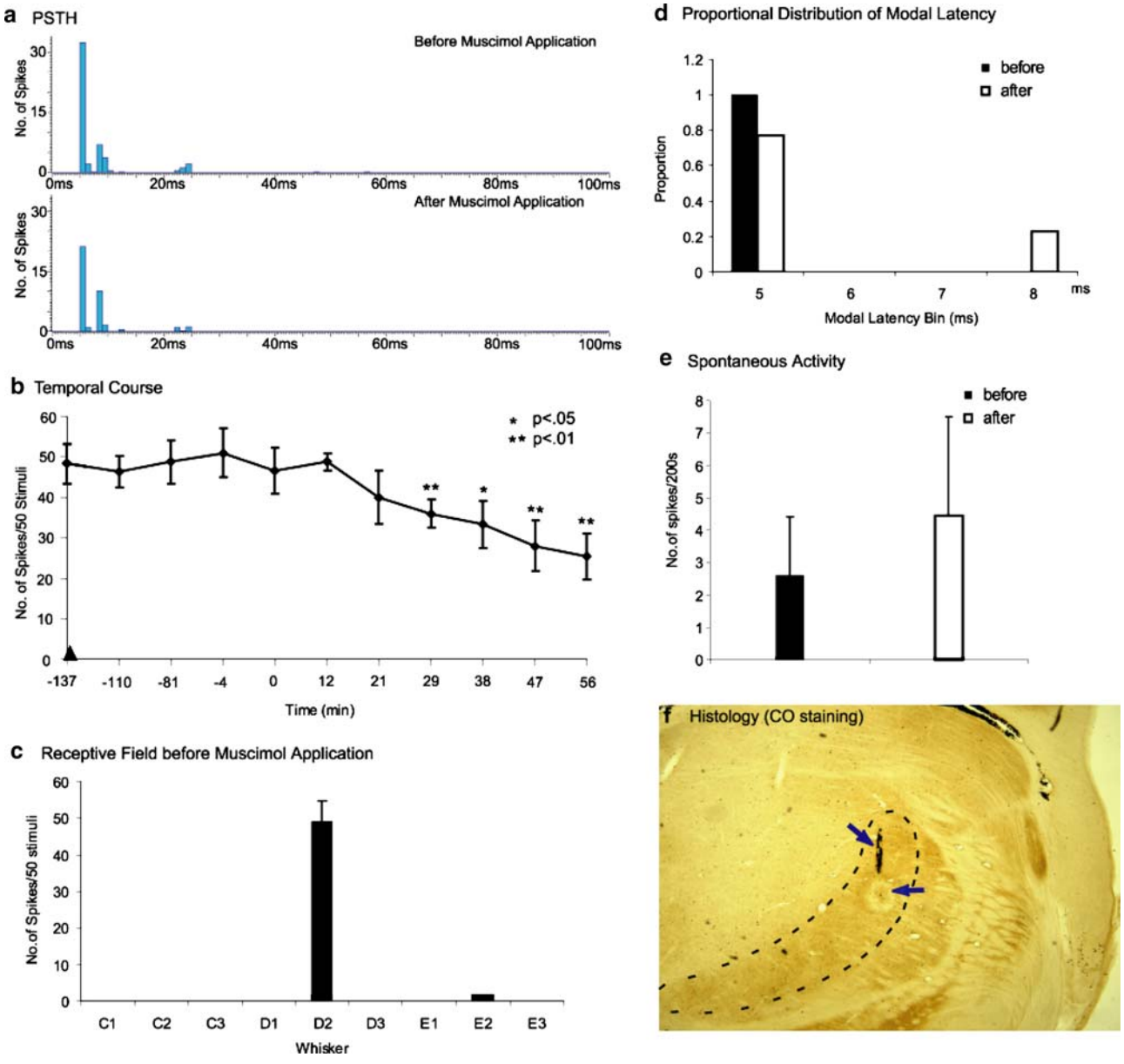

Fig. 4 Response changes of unit VPM04 after muscimol application. **a** Comparison of averaged PSTHs before and after muscimol application. *Top panel*: PSTH before muscimol application averaged from 650 trials during the 137-min period of baseline recording. *Bottom panel*: PSTH averaged from 1,300 trials after muscimol was applied. Note that overall changes occurred in both response magnitude and latency. **b** Time course before and after muscimol application. Response magnitude (not normalized) of unit VPM04 to stimulation of its principal whisker D2 was plotted against the time. The *black triangle* on the *x*-axis shows the last time of supplementary anesthetic injection. **c** RF of unit VPM04 before muscimol application mapped at 2T. The response magnitude (spike count/50 stimuli) was plotted for each whisker we tested. Note that unit VPM04 has single whisker RF, as did almost all of the VPM neurons studied. **d** Modal response latency shifted after muscimol application. Note the appearance of new modal latency at 8 ms after muscimol was applied. **e** The spontaneous activity did not change significantly after muscimol application. **f** Histology. The location of electrolytic lesion for VPM04 is shown in one coronal section after CO staining. The *left blue arrow* points to the electrode tract and the *right blue arrow* points to the electrolytic lesion. The VPMdm border was indicated by the *dashed line* (contrast was adjusted in Photoshop 6.0)

Hence muscimol application greatly changed the shape of the PSTHs of the same VPM neuron. No change in spontaneous firing rate was recorded before or after muscimol application.

Data from unit VPM02 is shown in Fig. 6. This unit represents another type of VPM neuron, histologically located near the tail region of VPM (Pierret et al. 2000) since it had a much larger RF size than VPM04 (Fig. 6c), but was still anatomically located within VPM (Fig. 6f). Muscimol also produced a response magnitude decrease and a modal latency shift (Fig. 6a). The time course of muscimol effects was very consistent in all

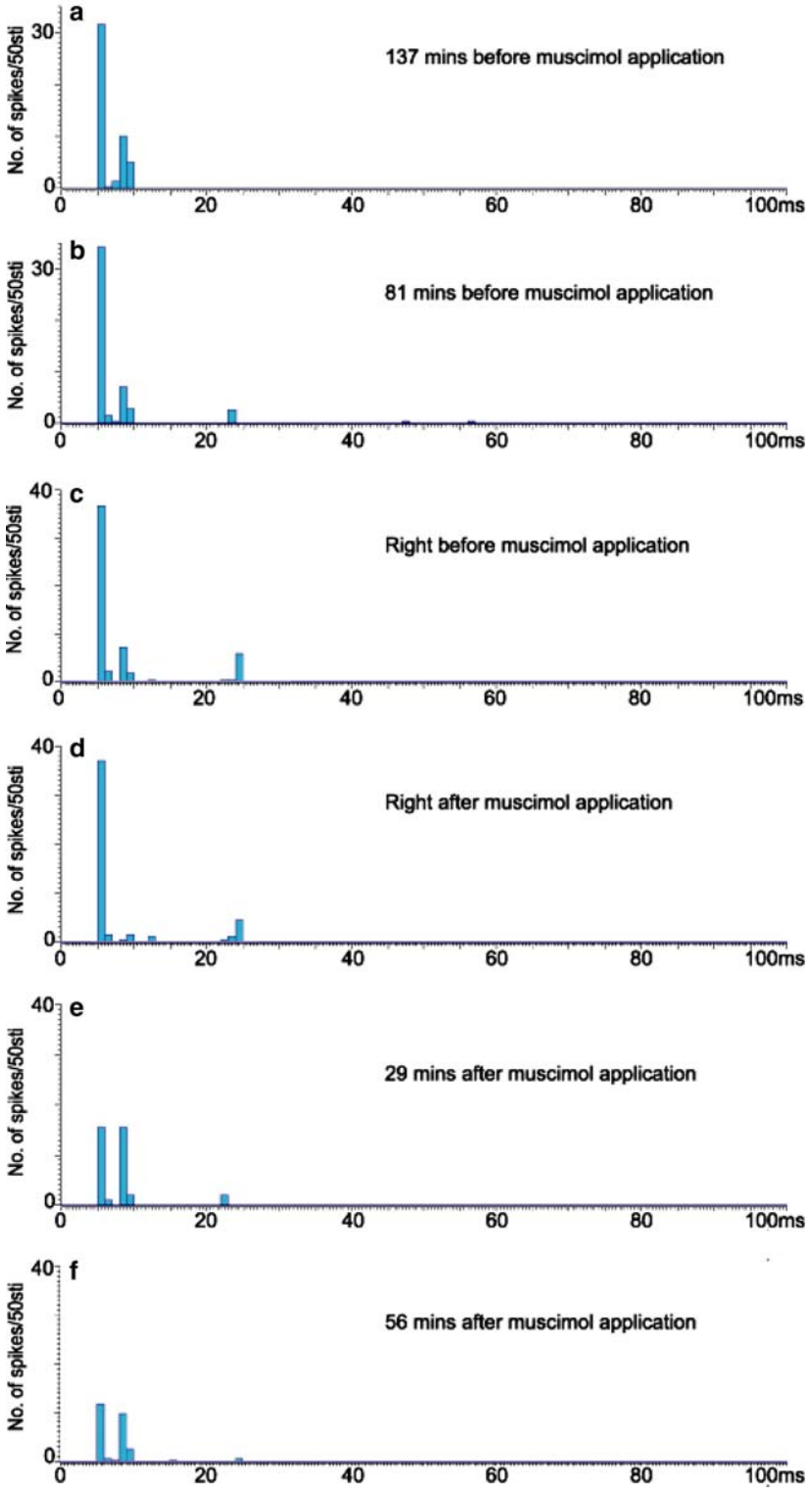

Fig. 5 Averaged PSTHs of unit VPM04 before and after muscimol application. **a** Averaged PSTH of neuron VPM04 from 150 trials collected 137 min before muscimol application. Response magnitude is 48.3 ± 2.8 spikes/50 stimuli (mean ± SEM, sampled at 2T stimulus intensity level, same for all panels). **b** Averaged PSTH from 200 trials at 81 min before muscimol application. Response magnitude is 48.8 ± 2.7 spikes/50 stimuli. **c** Averaged PSTH from 150 trials at 4 min before muscimol application. Response magnitude is 51.0 ± 3.5 spikes/50 stimuli. Note that the shape of the PSTHs before muscimol application is almost identical. **d** Averaged PSTH from 150 trials immediately after muscimol application (time 0). Response magnitude is 46.7 ± 3.3 spikes/50 stimuli. **e** Averaged PSTH from 200 trials at 29 min after muscimol application. Response magnitude is 36.0 ± 1.8 spikes/50 stimuli. **f** Averaged PSTH from 200 trials at 56 min after muscimol application. Response magnitude is reduced by roughly 50% (25.5 ± 2.8 spikes/50 stimuli). Note that muscimol effect gradually reduced the response magnitude and shifted the response latency

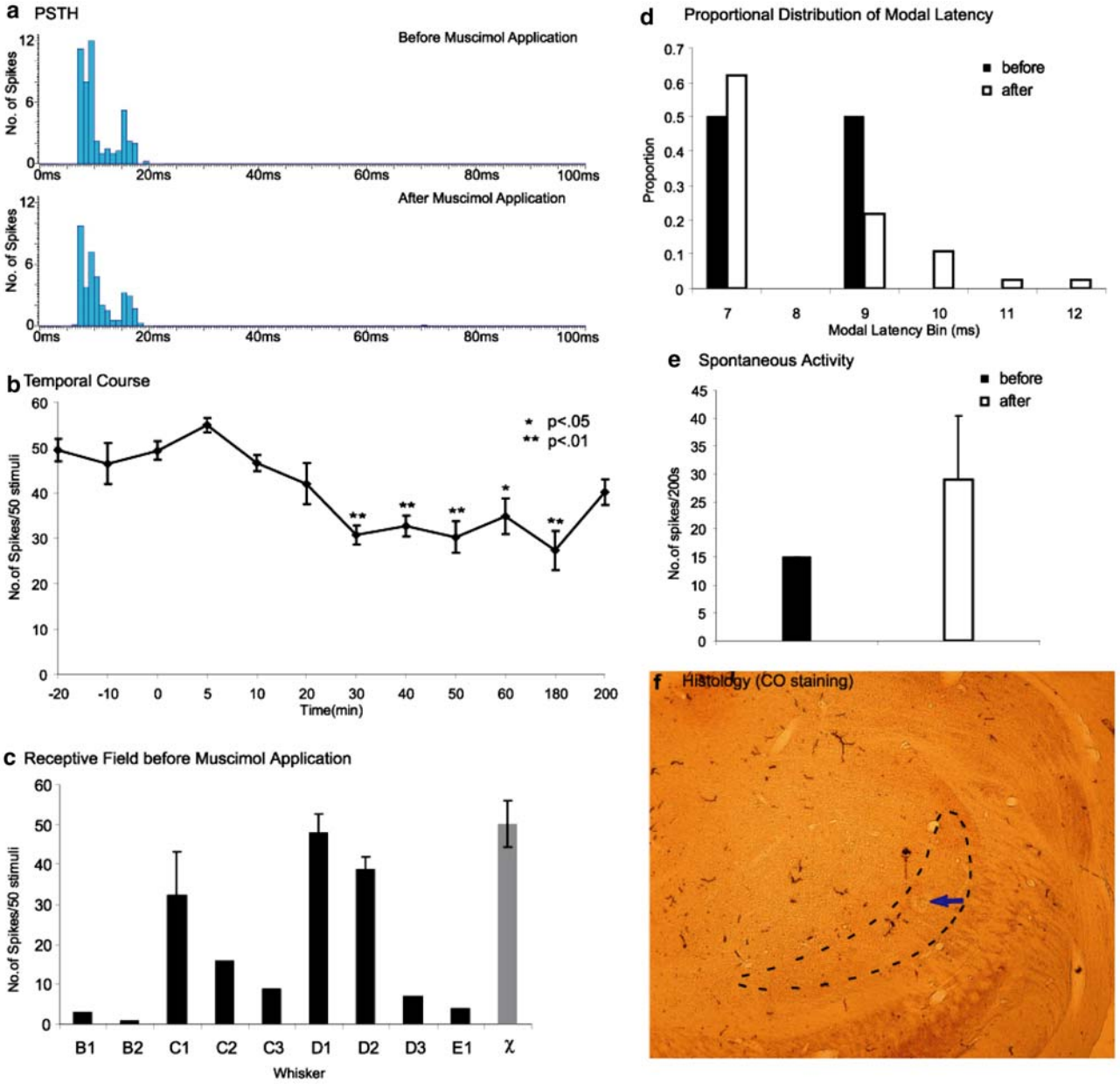

Fig. 6 Responses of unit VPM02 after muscimol application. **a** Comparison of averaged PSTHs before and after muscimol application. *Top panel*: PSTH before muscimol application averaged from 200 trials during the 20-min period of baseline recording. *Bottom panel*: PSTH averaged from 1,850 trials after muscimol was applied. **b** Time course of muscimol application. Response magnitude (unnormalized) of unit VPM02 to its principal whisker D1 stimulations was plotted against the time. No supplements were given since the rats did not show any apparent sign of changes in anesthesia depth after muscimol was applied. **c** RF of unit VPM02 before muscimol application mapped at 2T. The response magnitude (spike count/50 stimuli) was plotted for each whisker tested. Note that unit VPM02 has a multi-whisker RF. **d** Modal response latency in unit VPM02 showed a different pattern of latency shift after muscimol application. Note the appearance of new modal latencies >10 ms after muscimol application. These longer latency epochs were only present in this unit. **e** The spontaneous activity had no significant change after muscimol application. **f** Histology. The location of electrolytic lesion for VPM02 was visualized in one of the coronal sections after CO staining. The *blue arrow* pointed to the electrolytic lesion. The VPM border is indicated by the *dashed line*

VPM neurons. In unit VPM02, the first detectable response decrease occurred 30 min after muscimol was applied: the cell responded on average at 31 spikes to 50 D1 stimuli, which is only 64% ($P<0.002$) of its premuscimol value (Fig. 6b). Responses at 21 min postmuscimol time were also decreased but not significantly ($P<0.30$), which may be due to the variance commonly observed at this time point. The responses reduced further to 68% ($P<0.003$) at 40 min, 63% ($P<0.008$) at 50, 72% ($P<0.03$) at 60, 57% ($P<0.008$) at 180, and 84% ($P<0.08$) at 200 min, respectively (Fig. 6b). In this cell the modal response latency remained unchanged (Fig. 6d). Modal latency was changed in only 10% of the recording blocks after

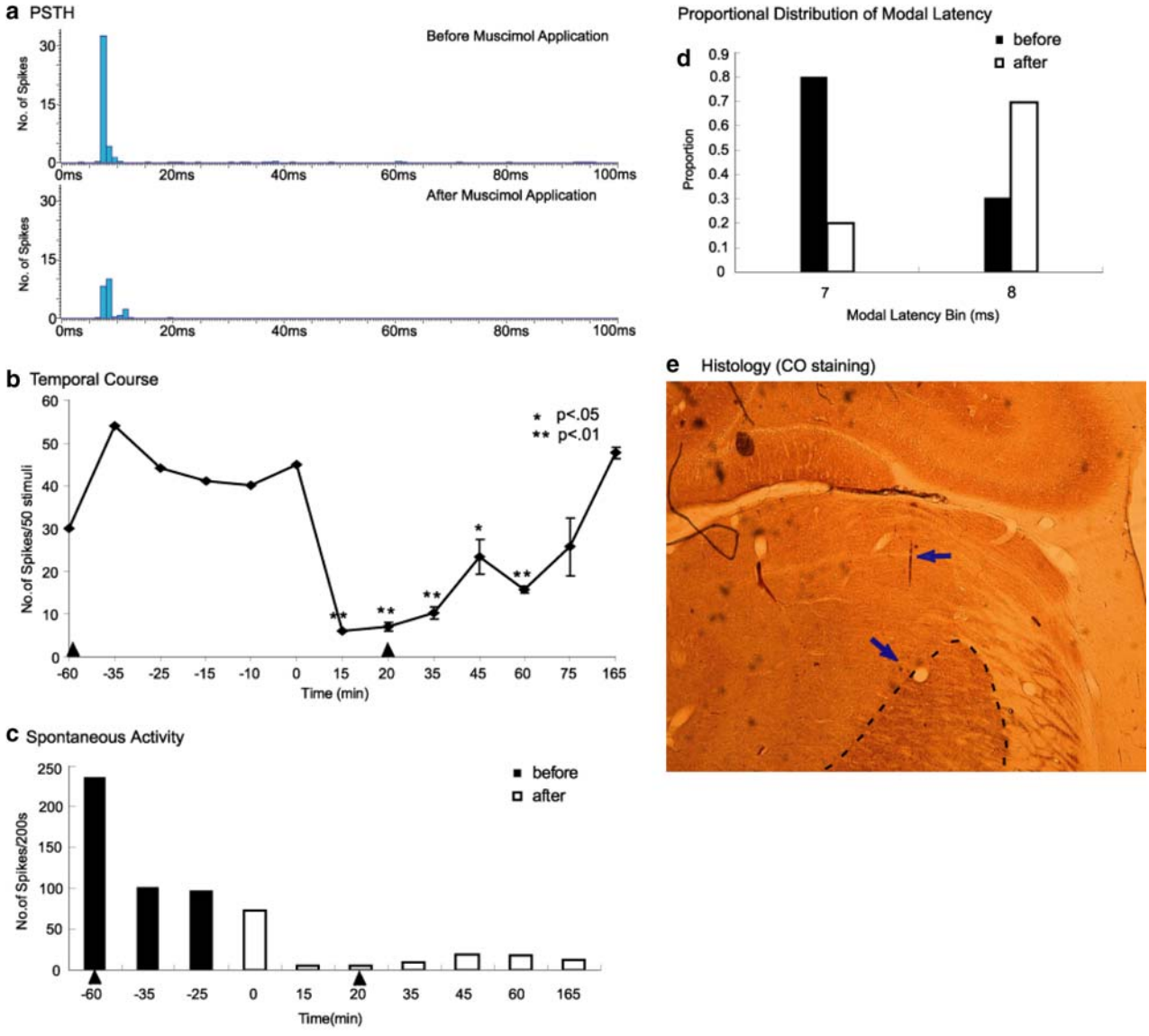

Fig. 7 Contralateral muscimol application has a greater effect on POm neurons than on VPM neurons. **a** Comparison of averaged PSTHs before and after muscimol application. *Top panel*: PSTH before muscimol application averaged from 250 trials during the 60-min period of baseline recording. The *bottom panel* was averaged from 1,000 trials after muscimol was applied. **b** Temporal course of the response magnitude change to "best whisker" (A4) stimulation before and after muscimol application. Receptive field was only mapped by a hand-held probe but not quantitatively. Time of anesthetic supplements is indicated by the *left black triangle* on the *x*-axis. The *black triangle on the right* indicates the wash-off time of muscimol by warm saline. **c** Change of spontaneous activity during the entire recording period. In contrast to VPM cells, there is a decrease in spontaneous activity of POm cells after the supplementary anesthetic injection that persists through the muscimol application period. **d** Modal latency before and after muscimol application. Response latency showed a clear shift to longer modal latency (8 ms) after muscimol. **e** Histological localization of the recording site lesion places the tip of the electrode in POm. The *left pointing arrow* indicates the electrode tract and the *right pointing arrow* shows the lesion location in the CO stained section. The *dashed line* shows the boundary of the VPM nucleus

muscimol application: however, the appearance of long latency is potentially important since long modal latency in lemniscal pathway cells may constitute a response modification. Seven out of eight VPM units showed latency shifts similar to unit VPM02 and VPM04.

The VPM responses were not changed in any of the cases in which muscimol was applied to the visual (VI) cortex in control animals (Fig. 3c).

Effects of muscimol application on POm neurons

Four units, whose response properties matched our unit-selection criteria for VPM neurons, were histologically located in the posterior nucleus (POm). POm neurons are also responsive to sensory inputs (Ahissar et al. 2000; Diamond et al. 1992a), but interact with barrel cortex differently than VPM neurons (Diamond et al. 1992b).

Data from these four neurons show that POm units decreased their response more quickly, and dropped their response magnitude more dramatically than the VPM neurons after contralateral muscimol application.

Changes in response of POm unit POM01 (Fig. 7e) were tested by stimulating the principal A4 whisker. Muscimol application almost eliminated responses to the A4 whisker within 15 min (Fig. 7b). Decrease of response magnitude and shift in response temporal structure is reflected in the shape changes of the PSTHs (Fig. 7a). Fifteen minutes after muscimol was applied, this unit changed from 42 spikes/50 stimuli to seven spikes per 50 whisker stimuli, which is only 14% ($P < 9\times10^{-4}$) of its normal value. The response was maintained near 16% ($P < 6\times10^{-4}$) at 20 min when muscimol was removed. These effects were reversible, in that 15 min after wash-off (second black triangle along the *x*-axis), the response began to recover. Two hours after muscimol wash-off, the response returned to its premuscimol level (Fig. 7b). The decrease of response magnitude in POm units was accompanied by a shift in modal latency. At 2T stimulus strength, POm neurons in four out of five trial blocks (80%) showed modal latencies of 7 ms, and one of five (20%) responded at 8 ms before muscimol application. After muscimol was applied, only 30% of trial blocks (7 of 23) retained a modal latency at 7 ms, while 70% (16 of 23) shifted to 8 ms (Fig. 7d). Muscimol application altered SA in some POm cells (Fig. 7c), but not others.

Muscimol application alters the response curves of VPM neurons

Normal VPM neurons in normal cortex on both sides (Fig. 2) showed increased response magnitude when the stimulus intensity increased above threshold, accompanied by shorter modal response latencies. The stimulus intensity–response magnitude curves in Fig. 2 indicate how VPM neurons respond to stimuli of different intensity, but neglect latency changes. We conclude that the response characteristics of one VPM neuron can be completely defined only when the information from the response magnitude is linked with the response latency. The data support the idea that response decreases, together with the elongation of the modal response latency after muscimol application, actually alters the threshold of VPM neurons, or to be more precise, alters the response curve of the VPM neurons. We re-measured the response curves in four of eight units after muscimol was applied. The intensity–response curves of these four units before and after inactivating the contralateral BFC are shown in Fig. 8a. In general, suppressing the contralateral BFC with muscimol produced consistently shifted response curves in all four neurons. For example, in unit VPM07, the threshold is 150 μm (blue solid line) before muscimol application. After inactivating the contralateral BFC, the threshold increases to 280 μm (blue dashed line). Muscimol also alters the slope of the response curve, and thus many fewer spikes were generated at 2T after muscimol application.

Other changes, such as unmasking of new units, were qualitatively noted. Units that were unresponsive before muscimol application began to respond to whisker stimulation after muscimol was applied. Appearance of new units in some recording sessions was highly correlated with contralateral cortical inactivation. Unmasking of a new unit was clearly observed by the appearance of a novel waveform on the reference oscilloscope, but could not be quantitatively measured using our experiment design.

Discussion

The present results document two new findings: first, activity in cortex on one side can influence thalamic relay neurons on the contralateral side, and second, cortex regulates thalamic relay neurons' responses by altering their response threshold and stimulus–response curve. Many previous reports have shown that responsiveness of thalamic relay neurons can be modulated by cortex (for review, see Castro-Alamancos 2004; Sherman and Guillery 1996, 2002; Sillito and Jones 2002). The current study provides evidence that thalamic relay neurons can be influenced from both hemispheres. The present data offers an explanation for the observation that after muscimol is applied to the BFC there are response decrements in the 3–10 ms component of responses of contralateral BFC neurons. The shortest latency (3–10 ms) responses in cortical neurons are usually ascribed to inputs from thalamic relay cells (Armstrong-James and Fox 1987; Armstrong-James et al. 1991). The functionally bilateral cortical activity could be the physical substrate for the rat to successfully perform perceptual tasks which require the bilateral integration of information sampled by the full array of whiskers (Krupa et al. 2001; Shuler et al. 2002). Our data indicate that an additional way for cortex to regulate the response properties of thalamic relay neurons is to alter their response threshold/sensitivity curve. The data suggest that cortex monitors thalamic activity very closely, and that the cortical regulation of thalamic relay neurons may not be solely achieved through lateral inhibition. Previous work suggests that the metabotrophic receptors play a role in such control (Castro-Alamancos 2004), however, further study is needed to clarify the individual molecular/cellular mechanisms for the threshold shifting seen in VPM neurons.

Methodological concerns

The most interesting finding in the present study is that VPM neurons change their responsiveness after silencing contralateral cortex. Muscimol has been widely used to reversibly block neural activity within a circumscribed region. Effects of muscimol have been proven to be well

localized and reversible when applied acutely, or chronically in auditory, somatic sensory, and visual cortex of bats, cats, and rats (Fox et al. 2003; Li et al. 2005; Krupa et al. 1999; Reiter and Stryker 1988; Zhang and Suga 1997). We mapped the effective area of muscimol suppression in vivo by electrophysiology, and the results are highly consistent with what had been reported using similar muscimol concentration in previous studies

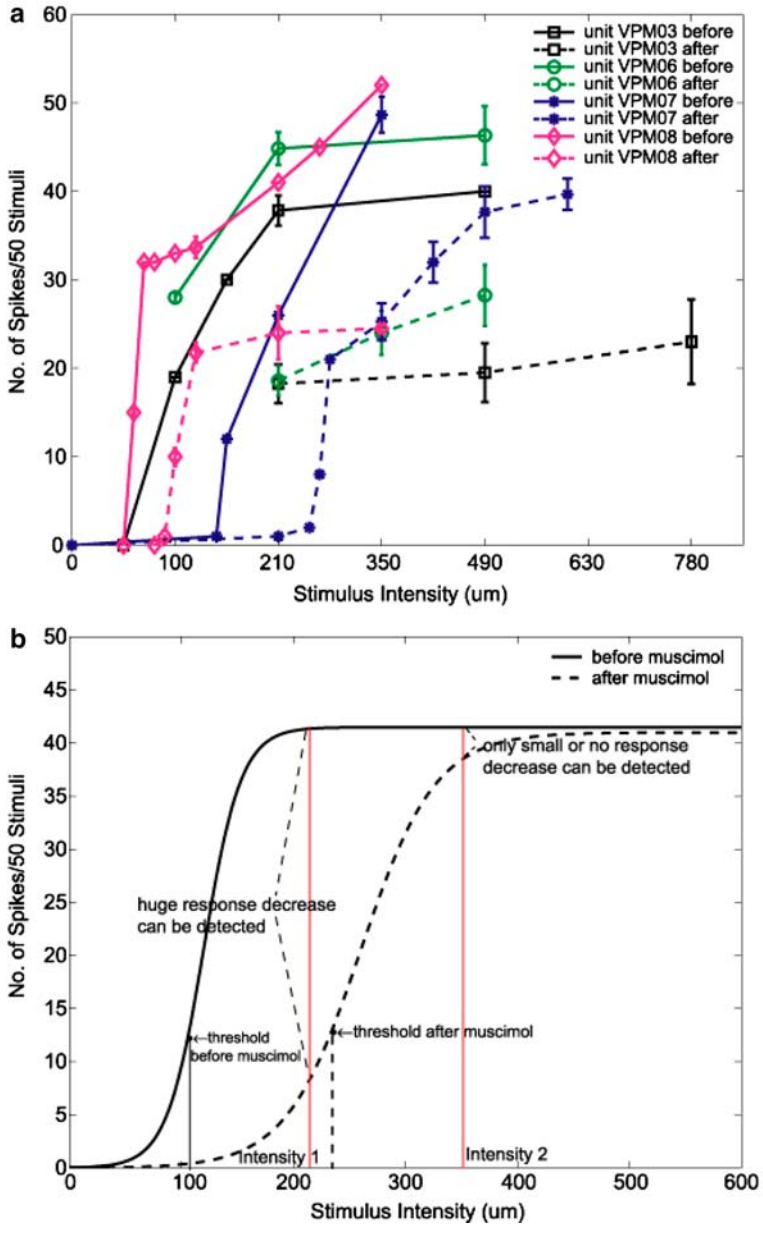

Fig. 8 Model of the muscimol-induced response curve changes. **a** Response curves shifted in VPM neurons ($n = 4$) after muscimol application. Response magnitude (spike count/50 stimuli) was plotted against the amplitude of whisker deflections (in μm). Data from the same VPM unit are rendered in the same color. The response curves of the same VPM neuron are shown before muscimol application (*solid lines*) and 60 min after muscimol application (*dashed lines*). Note that muscimol application systematically shifted the response curves of all four VPM neurons we tested. *Error bar*: SEM. **b** An hypothesis for how cortex could regulate the responsiveness of VPM neurons by shifting their response curves. *Solid* and *broken lines* represent the response curves of the same virtual VPM neuron when the cortex is intact (*solid line*) or inactivated (*dashed line*). We propose that the influence of cortical feedback is exerted through increasing or decreasing the sensitivity of the VPM neurons to ascending sensory inputs. This hypothesis highlights the idea that stimulus intensity level is a crucial variable when testing sensory neuron responses. For example, when stimuli are applied in the rising phase of the response curve (e.g., intensity 1 in the figure), response changes could be detected easily with the whisker stimulation paradigm. On the other hand, when test stimuli are applied at saturating levels of intensity (e.g., intensity 2 in the figure), the VPM neuron would be predicted to show small or no changes in the response magnitude (arbitrary scales were used for x and y axes). The present results would not be reproducible with stimuli of 0.5 mm or higher intensity

(Edeline et al. 2002; Li et al. 2005; Reiter and Stryker 1988; Wallace et al. 2001). The results of our acute muscimol suppression experiment showed that cortical activity was significantly suppressed (> 50%) by 10 mM muscimol solution within 20 min after application, as judged by reduced SA and smaller ERs to whisker stimuli in layer IV neurons in the D2 barrel. The gross effects of muscimol suppression recovered within 1 h after removal, consistent with previous studies using a similar experimental paradigm (Reiter and Stryker 1988). Although there is little question that muscimol suppresses neural activity in cortex, it is also the case in the acute experiment that muscimol could enter cortex along the electrode tract during direct application and alter the onset time of suppression by muscimol.

Thalamic relay neurons fire in two modes: tonic and burst (Lo et al. 1991; for review see Sherman 1996, 2001b). Tonic firing mode is thought to be more involved in information processing while bursting firing has been suggested to be a ''wake-up call'' stimulus to cortex (Sherman 2001a; for review see Sherman and Guillery 2002). It has been reported that inactivation of cortex shifts the firing mode of ipsilateral cat LGN neurons (Sillito and Jones 2002). In the present study all VPM neurons were operationally kept in the tonic firing mode by maintaining a steady depth of anesthesia and noting the interspike interval in the response. This also eliminated the confounding influences of recording condition changes. During our recording, no signs, such as voluntary whisker movement, response to squeezing the foot, or increase of spontaneous firing, were detected. And the fact that VPM and POm cells showed a different time course under the same experimental conditions diminished the possibility that the changes were induced by direct suppression of diffused muscimol. We also found that VPM neurons tended to fire more in bursts (or spindles) after long-term muscimol application and especially when stimulus intensities were well above 2T, which is consistent with what has been reported in the cat LGN (Sillito and Jones 2002).

Threshold determination

Neurons along the whisker–barrel pathway are sensitive to different stimulus parameters, such as amplitude, frequency, direction, velocity, and acceleration (Armstrong-James and Fox 1987; Gibson and Welker 1983a, b; Ito 1985, 1988; Lee and Simons 2004; Lichtenstein et al. 1990; Minnery and Simons 2003; Temereanca and Simons 2004; Waite 1973b; Wilent and Contreras 2004). Stimulus threshold has been investigated most frequently using long duration ramp-and-hold stimuli (Ito 1988; Lee and Simons 2004; Minnery and Simons 2003; Temereanca and Simons 2004). Stimulus amplitude interacts with velocity (Ito 1988; Waite 1973b; Wilent and Contreras 2004), and layer IV neurons are more sensitive to changes in stimulus amplitude than they are to velocity (Ito and Kato 2002), suggesting that VPM neurons also could be sensitive to stimulus amplitude. Furthermore, since we want to determine whether latency is shifted after inactivation of cortex, we chose to use a stimulus with short duration (3 ms) and a very brief rise time. Here we operationally defined response threshold as the lowest stimulus intensity that would cause the cell to generate action potentials at a criterion level above background. Small increases of around 10 μm in stimulus intensity can reproducibly move the VPM neuron from sub-threshold to threshold response levels (Fig. 2a, b). Our data indicate that this definition is very useful for comparing responses between cells in different animals. In this study VPM neurons showed quite a wide range of response thresholds, which could be due in part to differences in the driving power from the brainstem trigeminal complex. It is also important to emphasize that the VPM neurons were tested only by forward whisker deflections. In fact, VPM neurons show clear angular preferences (Simons and Carvell 1989; Timofeeva et al. 2003) and are organized topographically into an angular tuning map in VPM (Timofeeva et al. 2003). Thus, the variety of response thresholds can be partially due to the fact that not all VPM neurons analyzed in our experiments were tuned to forward whisker movements. However, since we did not map the angular tuning properties of each VPM neuron systematically, it remains unknown how the angular tuning preference interacts with muscimol suppression.

Each cell was tested at 2T intensity level, and from the response curves constructed in normal VPM neurons, it is clear that 2T is near a midpoint in the response range of VPM neurons. The response curves also emphasize that ''supramaximal'' stimuli similar to those applied in our previous studies and typically in studies from other laboratories, produce responses at saturation levels. It is likely that 2T stimulus intensity has relevance to natural stimuli encountered by a rat, but this statement requires careful documentation of natural stimuli. Our results support the idea that studies on cortical excitability should select stimulus parameters relevant to the experimental goal, since subtle thalamic and cortical response levels could depend on the stimulus configuration used to test the system. If the test stimuli were too high or too low the target neuron could be saturated or under-driven by the whisker stimulation as a result of excitability levels changing during the experiment. Therefore, caution is required when directly comparing the current results with others due to this significant feature of the experimental design. For example, VPM neurons have been shown to maintain their response latency to stimuli at different frequencies, which has been used as a cardinal characteristic of the nucleus (Ahissar et al. 2000). However, in those experiments VPM neurons were tested with ~2 mm whisker displacement produced by air-puff. This stimulus would be considered as very ''supramaximal'' under current experimental conditions. Thus, the differences in latency modulation identified in different studies can be attributed in part to the stimulus characteristics. Several

studies have shown a decrease in response latency with increases of stimulus intensity in thalamic (Ito 1988) and cortical neurons (Ito 1985; Wilent and Contreras 2004).

Corticothalamic projections: anatomy

All nuclei in the dorsal thalamus receive massive feedback projections from cortex. It has been reported that corticothalamic projections greatly outnumber the ascending sensory projections in the lateral geniculate nucleus (Guillery 1969; Liu et al. 1995). A better understanding of the structure of thalamo-cortico-thalamic circuitries is necessary to understand their functions in the working brain. As in other mammals, corticothalamic projections in rats mainly originate from pyramidal cells in layers V and VI (Hoogland et al. 1987; Land et al. 1995; for review, see Deschenes et al. 1998). These two groups of projections are distinguished by (1) their targets, (2) collaterals to TRN, (3) synapse morphology, and (4) axon branching patterns. The recipients of layer VI and V corticothalamic projections have been accordingly named as "first order" and "higher order" relay nuclei, respectively (Guillery 1995). Layer V projections are actually collaterals of corticobulbar or corticospinal fibers (Bourassa et al. 1995; Hoogland et al. 1987; Land et al. 1995). They always form small clusters of terminals, with larger terminal size in POm, and they do not innervate the TRN. On the other hand, projections from layer VI do send numerous collaterals to TRN (for review, see Deschenes et al. 1998). Layer V and VI projections accordingly differ substantially in function (for review, see Guillery and Sherman 2002; Sherman and Guillery 2002). The fine topography of CT projections from layer VI of BFC to VPM has been recently examined and discussed in detail (Deschenes et al. 1998). In brief, ipsilateral CT projections are highly ordered, and neurons in different sublayers [superficial section (VIa) vs. deeper sections (VIb) of layer VI] display different axon branching patterns in the thalamus. Axons from the upper section of layer VI obey a strict one-to-one relationship between barrels and barreloids while deeper cells tend to extend their collaterals to cover a much larger area in VPM and further invade into POm (Bourassa et al. 1995). Bilateral projections from cortex to thalamus do exist in rats. However, the reported projections do not originate from BFC and none terminate in VPM: the labeled fibers end in nuclei of the anterior group, medial dorsal, and submedial nuclei (Leonard 1969; Beckstead 1979; Kaitz and Robertson 1981; Reep and Winans 1982; Oda 1997). Thus, these bilateral projections are assumed to play only a limited role in the plasticity of sensory relay nuclei (VPM) after cortical manipulations in BFC. CT terminals form synapses on the distal part of the dendritic tree of VPM neurons, these synapses are glutamate gated which indicates the excitatory and regulatory nature of corticothalamic projections, while the fine-grained knowledge of the interconnections between layer VI, VPM and TRN is still incomplete. It is clear that cells in upper and lower sections of layer VI innervate thalamic relay nuclei differently, and identifying the functional implications of these differences in anatomy would constitute important future studies. We conclude that ipsilateral layer VI corticothalamic projections are the most likely substrate for the contralateral corticothalamic influences, and indeed Swadlow (1989) has reported an inhibitory effect of callosal stimulation on layer VI neurons in somatic sensory cortex of the rabbit.

Corticothalamic projections: function

Previous studies of different sensory systems and from various species have demonstrated that corticofugal projections are involved in the transmission mode change and/or the balance of center/surround components of the RF of thalamic relay neurons and in sleep/wake cycles (see Guillery and Sherman 2002; Sherman and Guillery 1996, 2002; Sillito and Jones 2002; Steriade and Timofeev 2003; Suga et al. 2003 for review). Available literature indicates a facilitatory role of CT fibers on thalamic neurons. Early studies in visual system show that the primary visual cortex influences the general excitability of thalamic relay neurons (Kalil and Chase 1970; Tsumoto et al. 1978). In bat's auditory system, MGN neurons shift their frequency tuning depending on the correspondence of the best frequency between cortical layer VI neurons stimulated and MGN neurons recorded (Yan and Suga 1996; Zhang et al. 1997; Yan and Suga 1998; see Suga and Ma 2003; Suga et al. 2003 for review). It has also been reported in the primate somatic sensory system, inactivation of ipsilateral area 3b either acutely or chronically with D-APV enlarges the RF size of thalamic neurons (Ergenzinger et al. 1998). Despite the numerous effects demonstrated, the detailed functions of CT projections still remain unclear. In rats, topically inactivating SI with lidocaine decreased the response of the majority of recorded VB neurons under anesthesia (Yuan et al. 1985) as well as in awake rats (Yuan et al. 1986). These results fit well with the synaptic arrangements of CT projections. CT projections influence lemniscal and paralemniscal neurons in rat somatic sensory system differentially since responsiveness of POm neurons decreased more after magnesium sulfate suppression of ipsilateral BFC under conditions where VPM neurons continued to fire (Diamond et al. 1992b). Other studies indicate VPM is affected by cortical feedback, for reversible inactivation of SI cortex immediately changed the spatiotemporal structure of RF in subtle ways (Krupa et al. 1999), and the effects can be nonlinear (Ghazanfar et al. 2001). Since these studies employed a method of global inactivation or removal of sensory cortex, instead of considering the possible dependence of observed thalamic response changes on the relationship of the tuning properties between thalamic neurons recorded and cortical neurons manipulated (Suga and Ma 2003), the

complexity of results reported may be simplified when the topography of cortex and VPM is considered. Temereanca and Simons (2004) recently reported that micro-stimulation of layer VI neurons in one barrel column increases the response magnitude of neurons in the corresponding barreloid to principal whisker stimulation, and at the same time reduces the responsiveness of cells in mismatched barreloids. These results demonstrate that matched cortical feedback exerts a topographic facilitatory influence on VPM neurons, and secondly, can sharpen the response possibly through mechanisms similar to Suga's "egocentric selection". The latter is a term arising from studies of auditory thalamus, and refers to a modulation in the best frequency of a subcortical neuron by its recent history of intense cortical input activity (Yan and Suga 1996; Zhang et al. 1997). The current concept in the somatic sensory system would be consistent with a similar cortical influence on sensory transmission through subcortical structures in other systems.

Cortical modulation of tuning properties of thalamic relay neurons

Various hypotheses had been proposed to explain thalamic and cortical interactions mostly based on lateral inhibition (Alitto and Usrey 2003; Krupa et al. 1999; Rauschecker 1998). We noticed that while lateral inhibition could account for the response magnitude change, it did not appear to explain the latency shift seen in the current study. Pharmacological activation of layer VI of barrel column with bicuculline selectively enhances the response of ipsilateral "matched" (homologous) barreloid neurons but suppresses those neurons in "non-matched" barreloids (Temereanca and Simons 2004). Their results convincingly demonstrate an important type of cortical modulation of "spatial tuning" (the RF characteristics) of VPM neurons, however, the underlying mechanisms still remain unclear. Our observations of the response curve shift after inactivation of cortex can be one of the possible ways that cortex regulates neurons at subcortical levels. Here we propose another way that cortex may regulate the responsiveness of VPM neurons, namely, by affecting their activation threshold (Fig. 8b). That is, the facilitatory role of increased cortical feedback is enacted through its influence on the response curves of VPM neurons. By modulating the threshold, VPM neurons can gate/filter certain information (Przybyszewski et al. 2000), and/or enhance/weaken the contrast between different stimuli (see Sillito and Jones 2002 for review). Threshold changes could also influence plasticity of VPM neurons after the cortical manipulations through latency as well as magnitude modulation. It is clear that in most cases corticothalamic modulation depends heavily on the topography of corticothalamic projections and on interactions between cortex, reticular nucleus, and thalamus, and the contribution of each of these parameters still need to be sorted out.

The response curves of VPM neurons could be viewed as the "stimulus intensity tuning curves". From this point of view, cortex unquestionably influences the "intensity tuning" of VPM neurons in addition to the "spatial tuning" reported by Temereanca and Simons (2004). Since neurons in the whisker to barrel pathway are tuned to several parameters such as the whisker specificity, amplitude (described in the present study), frequency and direction of whisker movements (angular tuning), many other tuning properties may be under cortical influence as well. Sillito et al. (1994) showed that corticofugal projections influenced LGN neurons by facilitating the synchronization of thalamic neurons with similar RF features as those of their cortical input cells. Experiments are ongoing to determine whether VPM cells lose their synchrony, or change their intrinsic pattern of synchronization, after the cortex on either side is silenced. These results are designed to address the unmasking effect: the appearance of newly responsive neurons that respond to whisker test stimulation only after contralateral muscimol application. This unmasking, together with the latency shift effect, may reveal additional features of the complicated influence of cortical feedback on transmission through the thalamus.

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