Direct Inhibition Evoked by Whisker Stimulation in Somatic Sensory (SI) Barrel Field Cortex of the Awake Rat

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INTRODUCTION

The cortical representation of each whisker in the rodent somatic sensory (SI) cortex has been referenced to clusters of neurons in layer IV (called barrels by Woolsey and Van der Loos 1970) that respond to deflection of whiskers on the contralateral face (Welker 1971). Extracellular recordings in awake-alert (Fanselow and Nicolesis et al. 1999; Fee et al. 1997; Nicolesis et al. 1995; Simons et al. 1992), awake-paralyzed (Simons 1978), and anesthetized (Armstrong-James and Fox 1987; Ito 1985; Welker 1971) rats show that neurons in barrels typically respond to principal whisker deflection by increasing their discharge rate 6–10 ms after the deflection. GABAergic inhibition typically follows the excitatory discharge thereby creating an “inhibitory trough” in the cumulative spike profile of a poststimulus time histogram (Carvell and Simons 1988; Simons 1978, 1985). Deflection of nonprincipal whiskers in the receptive field (RF) of cortical neurons, the excitatory “surround” whiskers, also increases cortical discharge rate, but at a longer latency and a lower magnitude than the principal whisker (Armstrong-James and Fox 1987). Using a two whisker stimulation paradigm Simons and colleagues have shown that surround whiskers can produce inhibitory interactions between neurons in adjacent barrels and septa (Brumberg et al. 1999; Simons 1985). The response evoked by principal whisker deflection is much reduced if an adjacent whisker is deflected 2–50 ms before the principal whisker (maximum inhibition ~20 ms). These results raise the possibility that stimulation of whiskers could directly inhibit the spontaneous discharge of cortical neurons under some conditions.

Intracellular recordings in vivo from anesthetized rats indicate that the initial response of barrel neurons to whisker deflection is typically an excitatory postsynaptic potential (EPSP) (Carvell and Simons 1988; Moore and Nelson 1998; Zhu and Connors 1999; Zhu and Sakmann 1998). EPSPs are followed by an inhibitory potential (IPSP); in fact EPSP-IPSP-EPSP sequences have been described in several cortical areas, including rat barrel cortex and cat SI whisker cortex (Hellweg et al. 1977; Zhu and Connors 1999; also see Kleinfeld and Delaney 1996). However, another sequence of events following whisker stimulation has also been described. An in vivo study using whole cell patch recording methods reported that 1 of 24 cortical neurons responded to a single whisker exclusively with IPSPs (Moore and Nelson 1998). Stimulation of thalamocortical fibers in brain slice preparations have also evoked solitay IPSPs in SI cortex (Agmon and Connors 1992). Here we present evidence from extracellular recordings in awake rats for suppression of spontaneous discharge following whisker stimulation, without any preceding excitation.

METHODS

All methods were approved by the University Animal Care Committee and were in accordance with NIH approved procedures.

Habituation to restraint

Male rats (n = 5) were handled every day for a week and placed on a reduced diet. Rats were habituated to being restrained by wrapping...
a towel around the animal and placing them in a loosely fitting cloth bag. The rat in the bag was slid into a loosely fitting plastic tube where they were offered chocolate milk during the restraint from a lick tube. Restraint was kept as short as possible, typically 20–30 min of licking chocolate milk; rats gained 20–30 g in a session. Once rats demonstrated that they would lie quietly and drink chocolate milk, they were prepared for surgery. Rats were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg) and a craniotomy was made over SI cortex. Five small holes were made in the skull, three over the cerebellum and two over rostral locations near the olfactory bulbs. Holes in the skull were tapped and blunt tipped screws were inserted. Using dental acrylic, a head post was fixed over the cerebellum (Bermejo et al. 1996) and a chamber was placed over the craniotomy. The chamber was sealed by a cap that could be unscrewed to give daily access to the exposed dura. Once animals recovered from the surgery, they were reacclimated to the restraint. Animals were monitored during head post restraint to see if they showed any signs of distress. Animals that frequently moved or made noises when their head was immobilized were eliminated from this study.

Recording

Rats were awake, quiet, and restrained during recording (Fig. 1). They were fed chocolate milk during the recording session between epochs of whisker deflection. Two miniature screw-advance microdrives were mounted into grids fitted over the chambers. When screwed onto the grids, the microdrives independently advanced single tungsten wire electrodes (FHC) into the cortex. At the outset electrode tips were separated by roughly 1 mm. As the electrodes penetrated through the dura, neuronal responses could be heard on an audio monitor in response to manual stimulation of the whiskers. For each electrode one whisker was selected as the principal whisker based on the response magnitude.

At the outset a period of spontaneous discharge was recorded for each neuron. The principal whisker for each electrode was deflected (~3 mm for 30 ms) at 1 of 8 stimulus frequencies (0.5, 1, 3, 6, 9, 12, 15, and 18 Hz) with an air puff stimulator constructed inhouse (James Long Company, Caroga Lake, NY). Data were collected for 50 s at all stimulus frequencies. Air puffs at each frequency in succession were directed from above the whisker pad. Whiskers were observed through an operation microscope throughout recording to ensure that only one whisker moved.

Electrodes were advanced in 75 μm steps. After cells in 3–5 depths had been sampled, lesions were placed at the bottom of each electrode penetration (3 μA, for 10 s). To distinguish between the two tracks, one of the two electrodes was advanced by 300 μm, the other was retracted by 300 μm, and a second lesion was made. The animal was killed with carbon dioxide, perfused with 0.1 M phosphate buffer, and the brain fixed with 4% paraformaldehyde. Once the brain sank in 30% sucrose, the cortex was removed, flattened between slides, and cut into 50 μm thick sections tangential to the cortical surface on a freezing microtome. Brains were stained for cytochrome oxidase (Wong-Riley and Welt 1980) and electrode tracks were reconstructed from serial sections. All data included in this paper are from animals with electrode tracks reconstructed and localized in the barrel field. In every case it was possible to specify whether a recording site was in a barrel or septum.

Data acquisition and analysis

A head stage (NB Labs) was used to connect a multichannel connector to the two electrodes on the rat’s head and to a multichannel neuronal spike data acquisition processor (Plexon, Dallas, TX). All waveforms from both electrodes were collected and saved for offline spike sorting. Units were rediscriminated offline using principal components and cluster cutting (Plexon). Stimulus evoked poststimulus

FIG. 1. Restrained rat preparation. The rat is restrained in a plastic tube with his head attached via the head post to a metal flange extending forward from the plastic tube. Two electrodes separated by a millimeter can be advanced separately using miniature screw advance microdrives.

FIG. 2. Autocorrelation histograms. Examples of 4 autocorrelations from 4 of the single units illustrated in the following figures. Bins are 1 ms. A and B: units whose responses are shown in Fig. 3. C and D: units shown in Fig. 5.
time histograms and raster displays were constructed from the spike trains of the discriminated units.

Stimulus

Air puffs controlled for force and duration were used for stimulating the whiskers. The air puff is a supramaximal stimulus with a ramp-and-hold puff of air (~200 mm/s).

STIMULUS LATENCY. Time 0 for the stimulus onset was triggered by a solenoid opening on the air line several feet from the animal’s face. Air travelling from the solenoid to the air outlet required 25 ms, and neuron response latency was calculated by subtracting 25 ms from the time to first spike for each trial.

RESULTS

The data presented here focus on cells that decreased their spontaneous discharge in a stimulus-linked fashion. In each animal, anywhere from 6 to 10 recording sites were tested, and...
at each site, multiple units were discriminated from two electrodes. A total of 48 units were discriminated of which 10 responded to whisker stimulation with I-only inhibition. Auto-correlation functions for four units discriminated from four separate electrodes in two animals are shown in Fig. 2. The striking feature of these recordings is that whisker stimulation inhibits cell discharge, without any detectable excitatory discharge. In all recordings, histology showed that the electrode was on a barrel edge or clearly in a septal zone (Figs. 3A, 4A, and 5A). Except for a single recording site where stimulation of a large mystacial whisker (C2) inhibited the spontaneous discharge, the rest of neurons in the sample responded to small whiskers (nose hair, D6, and D7) that the animal typically does not move much with suppression of spontaneous activity.

Stimulation of the C2 whisker at a frequency of 1 Hz inhibited the discharge of neurons recorded from electrode 2 (Fig. 3C, right PSTH) while the neuron on electrode 1 (Fig. 3C, left PSTH) was excited by C2 stimulation. Inhibition was restricted to the C2 whisker, as other whiskers (A2 and B1 are shown) did not evoke the same inhibition in the cortical cell.

In another animal, stimulation of the D6 whisker inhibited neurons on one electrode, while at the same time weakly exciting neurons on the other electrode (Fig. 4). Recording sites for the two electrodes were the edge of E7 whisker barrel and between C5, D6, and D7 barrels. One electrode had a receptive field of “F-row” whiskers that have no barrels associated with them, while the principal whisker for electrode 2 was the D6 whisker. At both recording sites on electrode 1, stimulation of the D6 whisker at 1 Hz inhibited the spontaneous discharge of neurons 1 mm lateral to the D6 barrel, on the edge of E7 barrel. Bin size 2 ms. Smoothing over 10 bins.
At the other recording sites in the same animal, the D7 whisker evoked either no response or an excitatory discharge. Inhibition is not restricted to mystacial whisker stimulation. Nose hair stimulation can also inhibit and excite neurons in the barrel field (Fig. 5C).

**Effect of stimulus frequency**

The neurons just described respond differently at higher stimulus frequencies. Figures 6, 7, and 8 show a more complicated pattern of modulation that develops at higher stimulus frequencies. The same neuron shown in Fig. 3 (electrode 2) shows a modulated pattern of discharge that is double the stimulus frequency at 12 Hz (Fig. 6). Inhibition begins 15 ms poststimulus and lasts 125 ms poststimulus. At higher stimulus frequencies, one-half (5 of 10) of the neurons show a switch from an apparent inhibitory trough to an excitatory discharge following the stimulus (Fig. 7). In the other neurons, there is a mixed excitatory/inhibitory response to the stimulus, where the neuron is inhibited for a large sequence of trials, but apparently cannot maintain this response to whisker stimulation (Fig. 6 and 8).
All recording sites were in SI barrel cortex, either on the edge of a barrel or in the septum between barrels. In 4 of 5 animals in which whisker stimulation evoked an inhibitory discharge, the most superficial recording sites were in layer II, III, or IV. In the remaining animal, whisker stimulation evoked an inhibitory discharge only in the deepest recording site in layer V.

**DISCUSSION**

The principal finding in this study was that whisker stimulation in the awake rat can block spontaneous discharge without evoking prior excitation. This suppression of discharge occurs at longer latencies than the fastest excitation produced at low stimulus frequencies. The cells showing I-only inhibition were found only in or at the border of septa.

Very early in the history of recording from single units in cat SI cortex, Mountcastle (1957) reported that stimulation with an air puff in the center of the receptive field evoked excitation, but stimuli directed outside of a neuron’s excitatory receptive field inhibited spontaneous discharge in cortical cells. An in vivo study carried out with intracellular methods in cat SI cortex (Hellweg et al. 1977) showed that the most frequent response to whisker stimulation was EPSPs followed by IPSPs. In the center of the receptive field where inhibition was the strongest, IPSPs occurred after excitation. Outside the center of the receptive field, however, IPSPs could precede EPSPs. Even further out in the receptive field, inhibition could occur as the only response to whisker stimulation. In this study no such gradation of inhibition has been detected. Specific whiskers inhibited the spontaneous discharge of neurons when other whiskers either evoked an excitatory discharge or had no effect.

In the rat whisker cortex, surround whiskers typically evoke an excitatory discharge with little evidence of an initial inhibition. In this study, we made no attempt to determine whether the entire receptive field of a neuron was inhibitory, but clearly the same neuron whose spontaneous discharge was inhibited could respond with an excitatory discharge to a neighboring whisker. A neuron whose response was suppressed by whisker stimulation could even generate an excitatory discharge to the same whisker when it was stimulated at a higher stimulus frequency.

A number of previous studies of the rat vibrissal cortex have described inhibition that follows excitation (Brumberg et al. 1996; Carvell and Simons 1988; Kyriazi and Simons 1993; Kyriazi et al. 1996; Simons 1985; Simons and Carvell 1989), and in addition, there is some evidence for I-only inhibition in cortical neurons (Hellweg et al. 1977; Moore and Nelson 1983).
The excitation-inhibition is very characteristic of the responses generated by VPM to barrel thalamocortical inputs when activated by the principal whisker. In the septa between layer IV barrels there is a dense input from the POm nucleus rather than VPM (Koralek et al. 1988; Lu and Lin 1993), and septal cells often respond equally strongly to more than one whisker (Armstrong-James and Fox 1987). The I-only inhibition has not been analyzed in the same detail, but its relatively long latency to onset is consistent with being generated by intracortical circuits rather than thalamocortical activation. An alternative explanation for both the postexcitatory inhibition and inhibition alone might be that at least part of the suppression of cortical discharge could be due to disfacilitation (the removal of drive onto the neuron) and not due to active inhibition (Contreras et al. 1996; Cowan and Wilson 1994). Arguments for this view are as follows: 1) input resistance of cortical neurons is higher during long-lasting hyperpolarizations evoked spontaneously or by thalamic stimulation and 2) GABAergic inhibitory mechanisms in cortex are relatively short compared with this inhibition. According to this view, active inhibition has a role in suppressing neuronal discharge, but this effect is short in duration, as short as the effect of excitation in increasing neuronal discharge. More important to this view, is the removal of all synaptic input, which increases the neurons input resistance and suppresses the neurons discharge. This study is an extracellular study and cannot distinguish between these possibilities.

Other studies

In this study the majority of neurons responded with excitation followed by inhibition and had similar characteristics to those reported previously by Simons, Carvell and colleagues (Brumberg et al. 1996; Simons 1985). Evidence of inhibition detected extracellularly can be seen 20 ms poststimulus and can last 100–150 ms (also see Kleinfeld and Delaney 1996). Inhibitory interactions in the rat vibrissal S1 cortex have been studied with a dual whisker stimulation paradigm to look at the effect of stimulating an adjacent whisker before, after, or during principal whisker stimulation. These studies have shown that as the number of stimulated adjacent whiskers increases, inhibition of principal whisker evoked responses increases (Brumberg et al. 1996). Adjacent whisker deflection within 10–20 ms suppresses the response of a neuron to principal whisker deflection (Simons 1985). One implication of these results is that whisker stimulation could have an inhibitory effect on neurons in barrel cortex. This study confirms that some septal neurons are directly inhibited by whisker stimulation. This study also suggests that the inhibitory effect of low frequency whisker stimulation can be seen even without dual whisker stimulation or the application of GABA agonists and antagonists.

Methodological issues

During whisker stimulation the rat can move its whiskers. It is possible that the whisker stimulus dependent inhibition described here is related to movement of the whiskers. However, this possibility can be ruled out at least for some of recording sites, like the nose hair, because the rat cannot move his nose hair voluntarily. Waxing and waning of attention as a cause of whisker stimulus related suppression is harder to rule out.
Implication

In most layers of barrel cortex, 15–20% of the neurons are GABAergic (Beaulieu 1993; Chmielowska et al. 1988; Lin et al. 1985). Layer IV is exceptional in that it contains a higher percentage of GABAergic neurons with estimates up to 50%. All neuronal elements in layer IV of mouse SI barrel cortex receive inhibitory input from GABAergic neurons in layer IV (Keller and White 1987). Both inhibitory and excitatory neurons receive thalamocortical excitatory inputs (White 1979, 1989), making the initial response to whisker deflection excitatory to all cell types, followed by a single synapse delay for the inhibition to take effect. Both recurrent excitatory and recurrent inhibitory connections within cortex likely play an important role in the generation of each cortical neurons response to whisker stimulation.

This study suggests that whisker stimulation can stop the firing of spontaneously active septal neurons. Behaviorally, one interpretation might be that when a rat’s nostril, nose hair, or particular whiskers are in contact with an object, input from other sensory inputs can be blocked at the cortical level. This instruction could operate to channel attention under behavioral conditions when a potential food source is close to or in contact with the nose (Brecht et al. 1997). The modulation of spontaneous neuronal discharge by inhibition might also help to synchronize the discharge of neurons at a particular frequency.

REFERENCES


