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MATCHING OF FEEDBACK INHIBITION WITH EXCITATION ENSURES FIDELITY OF INFORMATION FLOW IN THE ANTERIOR PIRIFORM CORTEX

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Abstract—Odor-evoked responses in mitral cells of the olfactory bulb are characterized by prolonged patterns of action potential (spike) activity. If downstream neurons are to respond to each spike in these patterns, the duration of the excitatory response to one spike should be limited, enabling cells to respond to subsequent spikes. To test for such mechanisms, we performed patch-clamp recordings in slices of the mouse anterior piriform cortex. Mitral cell axons in the lateral olfactory tract (LOT) were stimulated electrically at different intensities and with various frequency patterns to mimic changing input conditions that the piriform cortex likely encounters *in vivo*. We found with cell-attached measurements that superficial pyramidal (SP) cells in layer 2 consistently responded to LOT stimulation across conditions with a limited number (1–2) of spikes per stimulus pulse. The key synaptic feature accounting for the limited spike number appeared to be somatic inhibition derived from layer 3 fast-spiking cells. This inhibition tracked the timing of the first spike in SP cells across conditions, which naturally limited the spike number to 1–2. These response features to LOT stimulation were, moreover, not unique to SP cells, also occurring in a population of fluorescently labeled interneurons in glutamic acid decarboxylase 65-eGFP mice. That these different cortical cells respond to incoming inputs with 1–2 spikes per stimulus may be especially critical for relaying bulbar information contained in synchronized oscillations at beta (15–30 Hz) or gamma (30–80 Hz) frequencies.

Key words: olfactory, gamma oscillations, piriform cortex, inhibition, mitral cell.

INTRODUCTION

Cortical circuits are endowed with numerous sub-types of GABAergic interneurons that target both excitatory pyramidal cells, as well as other GABAergic cells. Within the primary olfactory, or piriform, cortex, several types of GABAergic interneurons have been identified based on their localization, anatomy, and biochemical properties (Ekstrand et al., 2001; Zhang et al., 2006; Young and Sun, 2009; Suzuki and Bekkers, 2010a,b; Bekkers and Suzuki, 2013). Targets of at least two of these cell-types in the anterior piriform cortex (aPC) have also been identified. GABAergic interneurons in layer Ia appear to provide inhibition on the dendrites of both superficial pyramidal (SP) cells located in layer 2 (L2) and another class of excitatory cells, the semilunar cells. In contrast, fast-spiking multipolar cells located in layer 3 (L3 FS cells) target inhibition onto the cell bodies of these same cells (Stokes and Isaacson, 2010; Suzuki and Bekkers, 2012).

At the level of action potential (spike) activity, somatic inhibition can profoundly impact the cortical response to mitral cell inputs arriving from the olfactory bulb. Following electrical stimulation of mitral cell axons in the lateral olfactory tract (LOT), this inhibition can shorten the duration of the evoked excitatory post-synaptic potential (EPSP) and spiking in superficial pyramidal cells (SP cells) (Tseng and Haberly, 1988; Luna and Schoppa, 2008). Inhibition acting to limit the duration of spiking has also been observed *in vivo*, during each cycle of odor-evoked gamma frequency (30–80 Hz) oscillations (Litaudon et al., 2008; Poo and Isaacson, 2009). Such a limited excitatory response could facilitate the SP cell response to prolonged incoming activity patterns from mitral cells (Margrie and Schaefer, 2003; Bathellier et al., 2008; Cury and Uchida, 2010; Patterson et al., 2013) that can include synchronized oscillations at the beta (15–30 Hz) or gamma (30–80 Hz) frequency (Adrian, 1950; Kashiwadani et al., 1999; Neville and Haberly, 2003; Martin et al., 2004; Beshel et al., 2007). A limited spike number following one round of input

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Abbreviations: aPC, anterior piriform cortex; DIG, digoxigenin; EGTA, ethylene glycol tetraacetic acid; EPSC/EPSP, excitatory post-synaptic current/potential; GAD67, glutamic acid decarboxylase 67; GAD65-eGFP, glutamic acid decarboxylase 65-enhanced green fluorescent protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IPSC, inhibitory post-synaptic current; L1,L2,L3, layer 1, layer 2, layer 3; L3 FS cell, Layer 3 fast-spiking cell; LOT, lateral olfactory tract; SP cell, superficial pyramidal cell; VGLUT, vesicular glutamate transporter.

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should allow SP cells to respond to subsequent inputs. It remains unclear, however, whether the ability of inhibition to limit spike number is robust. Such an effect of inhibition could be lost with different levels of LOT input, if inhibition and excitation scale differently with input strength from mitral cells, or during incoming stimulus patterns, if inhibition and excitation have differing short-term plasticity properties.

In this study, we performed patch-clamp recordings in slices of the mouse aPC to examine the impact of somatic inhibition on cortical responses while varying LOT stimulation intensity and stimulus patterns. Spike activity was recorded during cell-attached recordings, which was followed by whole-cell measurements of excitatory and inhibitory post-synaptic currents (EPSCs and IPSCs) to examine the mechanisms that shape the spike responses. Most recordings were in SP cells, but we also performed a subset of recordings in GFP-labeled cells in glutamic acid decarboxylase (GAD) 65-eGFP mice (Lopez-Bendito et al., 2004; Zhang et al., 2006). In both cell types, we found that a component of delayed inhibition likely derived from L3 FS cells consistently limited the spike response to 1–2 spikes per stimulus pulse across varying stimulus conditions.

EXPERIMENTAL PROCEDURES

All experiments were approved by the Institutional Animal Care and Use Committee at the University of Colorado Anschutz Medical Campus, in accordance with National Institutes of Health (NIH) guidelines.

Brain slice preparation and electrophysiology

Sagittal slices (300 μm) were prepared from the aPC of postnatal day 8–28 transgenic mice (male and female) that were heterozygous for GAD65-eGFP (B6CBAF1/J background; Lopez-Bendito et al., 2004). The slicing procedure was modified from that previously reported for preparation of olfactory bulb slices (Schoppa et al., 1998). Briefly, mice were anaesthetized with isofluorane and euthanized by decapitation. Brains were excised and placed in an ice-cold solution consisting of (in mM) 83 NaCl, 72 sucrose, 26 $NaHCO_3$, 10 glucose, 3.5 KCl, 3 $MgCl_2$, 1.25 NaH_2PO_4, and 0.5 $CaCl_2$. Brain hemispheres were sliced separately with a VT1000S or VT1200S vibratome (Leica, Buffalo Grove, IL, USA). After slicing, cortical slices were incubated in a solution consisting of (in mM): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, 3 KCl, 2 $MgCl_2$, 1 $CaCl_2$ (295 mOsm, pH 7.3 with 95% 0_2/5% CO_2 gas), and allowed to recover for 30–45 min at 33 °C. Brain slices were visualized with a 40× objective on an Axioskop 2 FS plus microscope (Carl Zeiss, Thornwood, NY, USA), equipped with differential interference contrast optics and an HBO 100 mercury lamp for fluorescence measurements. The aPC was identified based on its location relative to LOT and compact density of cells in L2. Experiments were conducted at 28 to 31 °C.

The base extracellular recording solution for the patch-clamp recordings contained (in mM): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, 3 KCl, 0.5–1.0 $MgCl_2$, 2 $CaCl_2$ (295 mOsm, pH 7.3 with 95% 0_2/5% CO_2 gas). Except where noted, the $GABA_B$ receptor antagonist CGP55845 (2–4 μM) was also added to the bath to minimize presynaptic inhibitory effects on glutamate release from associational fibers (Tang and Hasselmo, 1994; Franks and Isaacson, 2005). In parallel recordings, done with and without CGP55845, we found that the drug had no effect on the basic spike response in SP cells (compare Fig. 3B with its inset) nor on the onset- or rise-times of IPSCs ($n = 7$; $p > 0.48$ for both parameters). The internal pipette solution for cell-attached recordings contained 150 mM NaCl. For whole-cell recordings of EPSCs and IPSCs at different LOT stimulus intensities (Fig. 2), the internal solution included: 140 Cs-gluconate, 10 phosphocreatine, 10 TEA-Cl, 5 HEPES, 1 EGTA, 1 MgATP (280 mOSM, pH 7.2 with CsOH), along with 5 mM QX314 to block sodium channel-dependent action potentials. For other whole-cell recordings, the pipette solution contained (in mM): 125 K-gluconate, 10 HEPES, 1 EGTA, 2 $MgCl_2$, 0.025 $CaCl_2$, 2 NaATP, 0.5 NaGTP, and 5 QX314 (215 mOsm, pH 7.3 with KOH). Current and voltage signals were recorded with a MultiClamp 700B dual patch-clamp amplifier (Molecular Devices, Sunnyvale, CA, USA), digitized at 10 kHz, and filtered at 2.5–4 kHz. Data were acquired and analyzed with Axograph X (Axograph Scientific). We generally excluded whole-cell recordings from analysis if the test cells had resting membrane potentials more depolarized than −60 mV or if the series resistance during the recording obtained values of >20 MΩ.

Electrical stimulation was performed using a broken-tip patch pipette (4–6 μm) positioned directly on the LOT, always within 150 μm in the lateral direction with respect to the cell body of the test cells. A stimulus isolator (A365; World Precision Instruments, Sarasota, FL, USA) delivered single stimuli or stimulus patterns generated by a Macintosh G5 computer. Stimulus pulses/trains were delivered every 20–60 s to minimize current rundown. The stimulus intensity was varied between 10 and 200 μA, as indicated.

Most experiments involving a stimulus train applied to LOT were conducted at 100-μA intensity. The exceptions were the parallel recordings of IPSCs in SP cells and spiking in various classes of interneurons (Fig. 7C, D), which used weaker stimuli (30–80 μA). This was done in order to capture the complex facilitating-then-depressing dynamics of the IPSC response, which was to be compared to spikes in interneurons. Stimulus artifacts were blanked from the data traces in the figures.

For morphological analysis of SP cells and GAD65-eGGFP cells (Fig. 2A, Fig. 5A), 0.2% biocytin was included in the whole-cell patch pipette solution. Following fixation of slices in 4% formaldehyde, Cy5-conjugated streptavidin (1 μg/ml; Jackson ImmunoResearch, West Grove, PA, USA) was added. Cells were visualized under a confocal microscope (Olympus BX61WI FV1000 or the Olympus BX50 in the Rocky Mountain Taste and Smell Center at the University of Colorado Anschutz Medical Campus).

In the kinetic analysis of EPSCs and IPSCs, onset-times were estimated from the peak of the stimulation