Endogenous GABA and Glutamate Finely Tune the Bursting of Olfactory Bulb External Tufted Cells

Abdallah Hayar[1] and Matthew Ennis[2]

[1]*Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, Arkansas; and*
[2]*Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, Tennessee*

Hayar A, Ennis M. Endogenous GABA and glutamate finely tune the bursting of olfactory bulb external tufted cells. *J Neurophysiol* 98: 1052–1056, 2007. First published June 13, 2007; doi:10.1152/jn.01214.2006. In rat olfactory bulb slices, external tufted (ET) cells spontaneously generate spike bursts. Although ET cell bursting is intrinsically generated, its strength and precise timing may be regulated by synaptic input. We tested this hypothesis by analyzing whether the burst properties are modulated by activation of ionotropic γ-aminobutyric acid (GABA) and glutamate receptors. Blocking $GABA_A$ receptors increased—whereas blocking ionotropic glutamate receptors decreased—the number of spikes/burst without changing the interburst frequency. The $GABA_A$ agonist (isoguvacine, 10 μM) completely inhibited bursting or reduced the number of spikes/burst, suggesting a shunting effect. These findings indicate that the properties of ET cell spontaneous bursting are differentially controlled by GABAergic and glutamatergic fast synaptic transmission. We suggest that ET cell excitatory and inhibitory inputs may be encoded as a change in the pattern of spike bursting in ET cells, which together with mitral/tufted cells constitute the output circuit of the olfactory bulb.

INTRODUCTION

Olfactory information is encoded, in part, by oscillating neural assemblies (Kauer 1998; Kay and Laurent 1999; Laurent et al. 1996; Wehr and Laurent 1996). Oscillations have been linked to bursting and synchronization of neuronal activity (for review, see Llinas 1988), which may contribute to information coding. External tufted (ET) cells, which are located at the first site of synaptic processing in the olfactory system, generate rhythmic spike bursts at frequencies associated with rodent sniffing. Early intracellular recordings in vivo indicated that olfactory nerve and odor stimulation produced bursts of action potentials in some juxtaglomerular cells (Getchell and Shepherd 1975; Wellis and Scott 1990). In olfactory bulb slices, ET cells exhibit spontaneous spike bursts that persist and become more regular in the presence of fast synaptic blockers, which include the non–*N*-methyl-D-aspartate (NMDA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), the NMDA receptor antagonist ($\pm$)-2-amino-5-phosphopentanoic acid (APV, 50 μM), and the γ-aminobutyric acid type A ($GABA_A$) receptor antagonist gabazine (SR95531, 10 μM) (Hayar et al. 2004a). These results suggest that fast synaptic transmission might not be required for bursting; however, it may regulate the timing of the occurrence of the bursts. Indeed our recent study (Hayar et al. 2005) showed that the bursting activity of ET cells that are

associated with the same glomerulus may be synchronized by coincident excitatory input as well as correlated bursts of inhibitory inputs.

The roles of ionotropic glutamatergic and GABAergic synaptic input in regulating the temporal structure of ET cell bursting discharge are unknown. ET cells receive glutamatergic synapses from olfactory nerve terminals and GABAergic synapses from periglomerular (PG) cells, which receive direct excitatory input from ET cells (Hayar et al. 2004b). Olfactory nerve terminals may initiate synchronized activity within a single glomerulus by providing a common excitatory input to ET cells, whose dendrites extensively ramify throughout a single glomerulus (Hayar et al. 2004a). Moreover, periglomerular (PG) cells, which greatly outnumber ET cells, provide a powerful synchronized feedback inhibition to ET cells. Such inhibition is likely to play a role in regulating the temporal dynamics of ET cell spike bursts. By adjusting the amplitude and timing of spike bursts, or the number of spikes per burst, synaptic inputs can exert characteristic changes in operation of the glomerular network.

The purpose of this study was to investigate the impact of fast excitatory and inhibitory synaptic inputs on the bursting of ET cells. We therefore examined the changes in spike dynamics that are produced by agonists and antagonists of ionotropic GABA and glutamate receptors. Our results indicate that the timing of bursts and the number of spikes in bursts, which are among the most important determinants of ET cell output, are constantly adjusted by activation of GABA and glutamate receptors.

METHODS

Sprague–Dawley rats (21–29 days old), of either sex, were decapitated in accordance with Institutional Animal Care and Use Committee and National Institutes of Health guidelines. The olfactory bulbs were removed and immersed in sucrose–artificial cerebrospinal fluid (sucrose-aCSF) equilibrated with 95% O_2-5% CO_2 (pH = 7.38). The sucrose-aCSF had the following composition (in mM): 26 $NaHCO_3$, 1 NaH_2PO_4, 2 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 20 glucose, 0.4 ascorbic acid, 2 sodium pyruvate, and 234 sucrose. Horizontal slices (400 μm thick) were cut with a microslicer (Ted Pella, Redding, CA). After a period of recovery at 30°C for 15 min, slices were then incubated at room temperature (22°C) in aCSF equilibrated with 95% O_2-5% CO_2 [composition in (mM): 124 NaCl, 26

NaHCO$_3$, 3 KCl, 2 MgCl$_2$, 2 CaCl$_2$, 0.4 ascorbic acid, 2 sodium pyruvate, and 20 glucose] until used. For recording, a single slice was placed in a recording chamber and continuously perfused at the rate of 1.5 ml/min with normal aCSF equilibrated with 95% O$_2$-5% CO$_2$. All recordings were performed at 30°C. Neurons were visualized using an upright microscope (Olympus BX50WI, Tokyo, Japan) equipped with differential interference contrast optics.

Extracellular recordings (loose patch) were obtained using pipettes (resistance: 5–8 MΩ) filled with aCSF. Pipettes were pulled from borosilicate glass capillaries with an inner filament (1.5-mm outer diameter; Clark, Kent, UK) on a pipette puller (P-97, Sutter Instrument, Novato, CA). Recordings were made in voltage-clamp mode using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Drugs and solutions of different ionic content were applied to the slice by switching the perfusion with a three-way electronic valve system. Gabazine (SR95531), CNQX, and APV were purchased from Research Biochemicals International (Natick, MA). The concentrations of blockers used in this study (APV, 50 μM; CNQX, 10 μM; gabazine, 10 μM) have been shown previously to effectively and selectively block NMDA, non-NMDA, and GABA$_A$ receptors, respectively, in juxtaglomerular neurons (Hayar et al. 2004b, 2005). Analog signals were low-pass filtered at 2 kHz (Axopatch 200B) and digitized at 5 kHz using a Digidata-1322A interface and pClamp9 software (Molecular Devices). Spike detection was performed off-line using the Mini analysis program (Synaptosoft, Decatur, GA). The times of occurrence of events were stored in ASCII (American Standard Code for Information Interchange) files and imported into Origin 7.0 (Microcal Software, Northampton, MA) for further analysis using algorithms written in LabTalk. Custom-designed computer algorithms were used off-line to evaluate the pattern of bursting of spikes throughout the experiments by measuring the following parameters: overall firing frequency, bursting frequency (defined as the number of bursts per second), spikes per burst, intraburst frequency (defined as the frequency of spikes within a burst), and burst duration (defined as the time interval between the first and the last spike in a burst). A burst of spikes was defined as a series of two or more consecutive spikes that had interspike time intervals of <75 ms (i.e., an interspike time interval of >75 ms was considered to signal the beginning of another burst). A moving average of each parameter (>1 min) was also computed to dampen fast variations and to evaluate the mean of each parameter in different conditions. Data, expressed as means ± SE, were statistically analyzed using paired t-tests (Origin 7.0 software) or one-way repeated-measures (RM) ANOVAs followed by post hoc comparisons of individual burst properties using Newman–Keuls (N-K) test (SigmaStat software), as indicated in the text. Any difference in distributions was analyzed using Kolmogorov–Smirnov test (K-S test, Clampfit 9.2 software).

RESULTS

ET cells are the only juxtaglomerular cells that exhibit spontaneous spike bursting; they are readily identified in extracellular recordings and their activity is stable and can be monitored for prolonged periods of time, whereas bursting undergoes rundown in whole cell recordings (Hayar et al. 2004a). We have previously shown (Hayar et al. 2004a) that bursting in ET cells becomes more regular when fast synaptic transmission is blocked with APV, CNQX, and gabazine, referred to here as fast synaptic blockers. The following experiments investigate in detail how endogenous glutamate and GABA modulate the properties of the spike bursts.

Blocking fast synaptic transmission weakens bursting by decreasing the number of spikes per burst

We first tested the effect of blocking both excitatory and inhibitory fast synaptic transmission on the bursting properties of six ET cells. In particular, we tested whether the strength of bursting (evaluated as the number of spikes per burst) could be modulated by fast synaptic input. We also quantified several other parameters such as burst duration and the frequencies of spikes, bursts, and spikes within the bursts (see METHODS for details). Simultaneous application of CNQX, APV, and gabazine in six ET cells (Figs. 1 and 3) reduced the number of spikes per burst from 5.2 ± 0.4 to 3.9 ± 0.4, the overall firing frequency from 9.2 ± 0.9 to 6.3 ± 0.8 spikes/s, and the intraburst frequency from 102 ± 13 to 75 ± 12 Hz ($P < 0.01$, paired t-test). In contrast, the fast synaptic blockers did not significantly change the burst duration (control: 42 ± 3 ms; blockers: 40 ± 3 ms, $P = 0.13$, paired t-test) or the interburst frequency (control: 1.8 ± 0.2 bursts/s, blockers: 1.7 ± 0.2 bursts/s, $P = 0.30$, paired t-test). These results confirm our previous findings that fast synaptic blockers do not change the burst frequency (Hayar et al. 2004a) and extend these findings by showing that synaptic blockers significantly reduce the number of spikes per burst.

Blocking fast inhibitory synaptic transmission enhances bursting by increasing the number of spikes per burst

The previous results do not distinguish whether GABA and glutamate exert similar or opposing modulatory actions on bursting. We therefore investigated the effect of separately blocking inhibitory and excitatory fast synaptic transmission on the bursting properties of 12 other ET cells. Because inhibitory input to ET cells is greatly reduced during blockade of excitatory synaptic transmission (Hayar et al. 2005), we examined first the effect of blocking inhibitory transmission and, subsequently, the effect of additional blockade of excitatory transmission (Figs. 1 and 3). In six of 12 ET cells tested, application of gabazine (10 μM) produced a transient increase in overall firing (due to an increase in burst frequency lasting 1–2 min at ~3 min from beginning of application), which was followed by a return to control state. The transient increase in burst frequency was typically associated with a transient decrease in the number of spikes per burst (Fig. 1B), as observed when ET cells were depolarized with current injection (see Fig. 5 of Hayar et al. 2004a). This transient effect was excluded from subsequent analysis because it was never observed when gabazine was applied together with APV and CNQX ($n = 6$, previous section), suggesting that it may reflect excessive network activity due to disinhibition of glutamatergic presynaptic input to ET cells. In control conditions, ET cells exhibited a variable number of spikes per burst (2 to 12 spikes/burst). Application of gabazine (10 μM) shifted the distribution of number of spikes per burst toward larger values in all ET cells tested ($n = 12$, $P < 0.001$, K-S tests). The mean number of
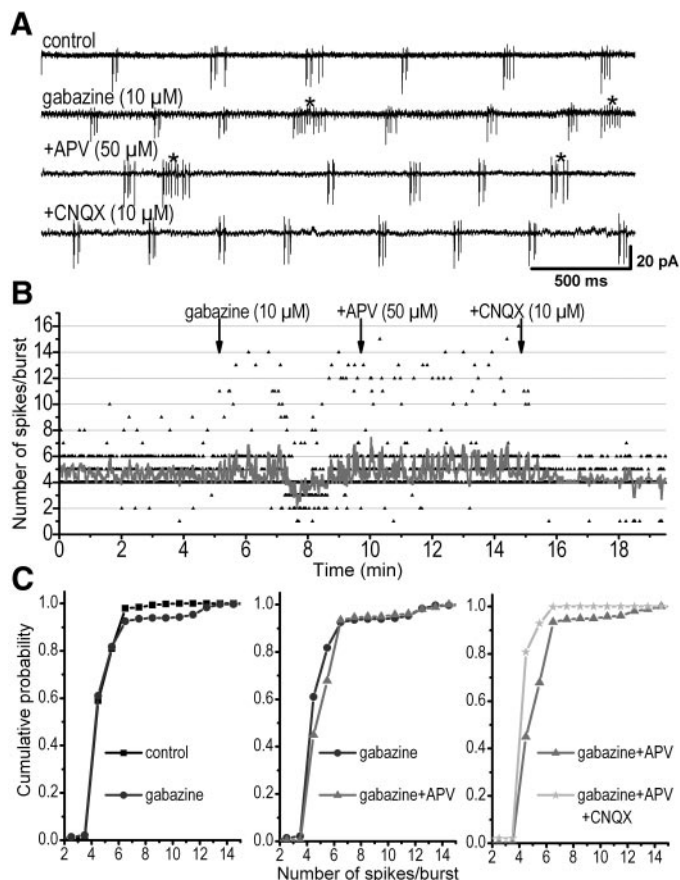
FIG. 1. Effect of inhibitory and excitatory fast synaptic blockers on bursting. *A*: extracellular recording from an external tufted (ET) cell in control and during subsequent application of gabazine (SR95531), (±)-2-amino-5-phosphopentanoic acid (APV), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Note that application of gabazine induced the appearance of bursts with relatively larger number of spikes (asterisks). Application of CNQX eliminated these large bursts and reduced on average the number of spikes/burst. *B*: scatterplot and a running average (gray line) of the number of spikes/burst during a typical experiment where the synaptic blockers were applied consecutively. *C*: cumulative probability histograms of the distribution of the number of spikes/burst in control and during application of gabazine, APV, and CNQX. Application of gabazine (10 μM) shifted the distribution of number of spikes/burst toward larger values [*left*, $P < 0.001$, Kolmogorov–Smirnov (K-S) test]. In the presence of gabazine and APV, application of CNQX (10 μM) significantly shifted the distribution of the number of spikes/burst toward lower values (*right*, $P < 0.001$, K-S test). Data in *A–C* were obtained from the same ET cell.

spikes per burst was slightly increased by gabazine from 4.6 ± 0.4 to 5.5 ± 0.4 bursts/s ($n = 12$, $P < 0.01$, one-way RM ANOVA followed by N-K test). The overall firing frequency was also significantly increased from 7.7 ± 1.4 to 8.5 ± 1.4 Hz ($n = 12$, $P < 0.01$, one-way RM ANOVA followed by N-K test). These findings indicate that tonic activation of GABA$_A$ receptors regulates the number of spikes per burst, but does not control the bursting frequency or duration.

GABA$_A$ receptor activation inhibits bursting or reduces the number of spikes per burst

In contrast to glutamate, it is unknown how GABA affects the excitability of ET cells through GABA$_A$ receptors. The finding that gabazine increases the number of spikes per burst (see above) suggests that endogenously released GABA, might

serve to weaken the burst by limiting the number of spikes per burst. This hypothesis was further tested by examining the effect of the GABA$_A$ receptor agonist isoguvacine (10 μM) on the bursting properties of six ET cells in the presence of APV and CNQX. In seven of 10 cells, isoguvacine inhibited bursting completely, whereas in the remaining three cells, isoguvacine decreased the number of spikes per burst by $52 \pm 1.5\%$ (from 4.3 ± 1.2 to 2.1 ± 0.7 spikes/burst, $n = 3$ of 10, paired t-test, $P < 0.05$, Fig. 2) without significantly changing the bursting frequency (control: 5.2 ± 1.2 bursts/s, isoguvacine: 4.8 ± 1.2 bursts/s, $n = 3$ of 10, paired t-test, $P = 0.8$). In the presence of CNQX, APV, and gabazine, isoguvacine (10 μM) produced no effect on the burst properties ($n = 4$; $P > 0.05$, paired t-test), indicating that the inhibitory effects of isoguvacine were due to activation of GABA$_A$ receptors. These results suggest that that activation of GABA$_A$ receptors might inhibit or shunt the bursting of ET cells.

Blocking excitatory fast synaptic transmission reduces the strength of bursting

In the presence of gabazine, additional application of APV (50 μM, $n = 12$) did not have a significant effect on any of the bursting parameters studied (Figs. 1 and 3). In the presence of gabazine and APV, application of CNQX (10 μM) significantly shifted the distribution of the number of spikes per burst toward lower values ($n = 12$, $P < 0.001$, K-S test, Fig. 1*C*) and significantly reduced the mean number of spikes per burst from 5.2 ± 0.4 to 3.3 ± 0.4 ($n = 12$, $P < 0.001$, one-way RM ANOVA followed by N-K test). Moreover, CNQX reduced the overall firing frequency from 8.0 ± 1.1 to 5.8 ± 1.2 spikes/s ($n = 12$, $P < 0.001$, one-way RM ANOVA followed by N-K test), the burst duration from 48 ± 5 to 33 ± 4 ms ($n = 12$, $P <$
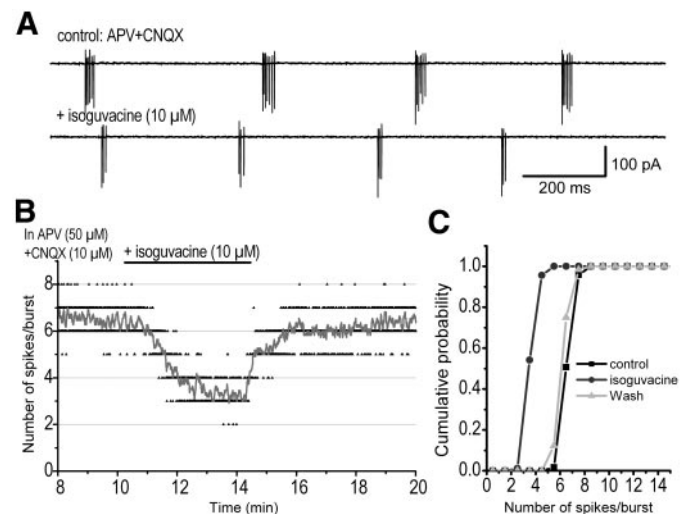

FIG. 2. Effect of activation of γ-aminobutyric acid type A (GABA$_A$) receptors on ET cell bursting. *A*: extracellular recording from an ET cell showing the effect of the GABA$_A$ receptor agonist isoguvacine, applied in the presence of CNQX and APV. Note the reduction in the number of spikes/burst without a significant change in the bursting frequency. *B*: scatterplot and a running average (gray line) of the number of spikes/burst during a typical experiment in which isoguvacine was applied. *C*: cumulative probability histograms of the distribution of the number of spikes/burst in control, during application of isoguvacine and after washout of the drug. Isoguvacine induced a significant reduction in the number of spikes/burst ($P < 0.0001$, K-S test). Data in *A–C* were obtained from the same ET cell.
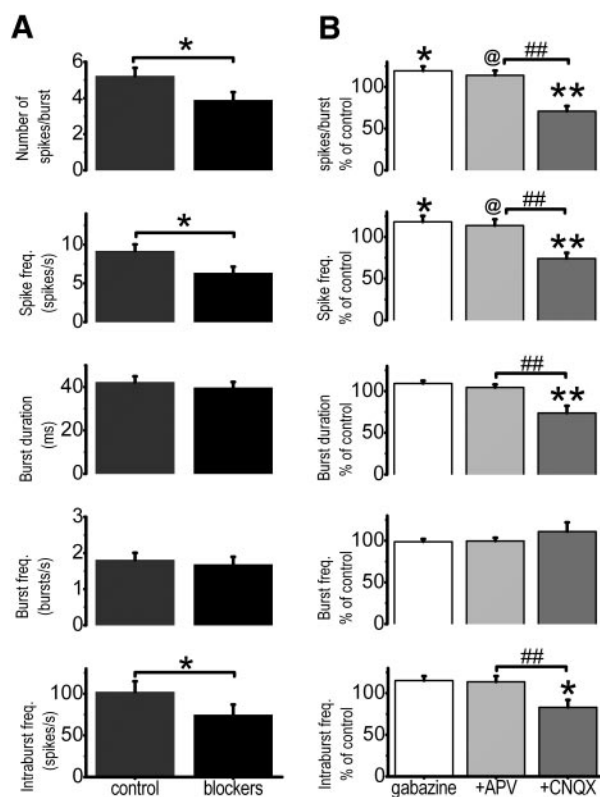
FIG. 3. Effect of inhibitory and excitatory fast synaptic blockers on bursting. *A*: grouped data showing changes in the burst properties that occurred during application of the fast synaptic blockers (APV, CNQX, and gabazine) when applied together ($n = 6$; $*P < 0.01$, paired *t*-test). *B*: grouped data showing changes (expressed as percentage of control) in the burst properties that occurred during cumulative application of the synaptic blockers (*1st column*: gabazine; *2nd column*: gabazine + APV; *3rd column*: gabazine + APV + CNQX) in a different group of ET cells ($n = 12$; $@P < 0.05$, $*P < 0.01$, $**P < 0.001$ compared with control; $##P < 0.001$ compared with respective preblocker baseline; one-way repeated-measures ANOVA followed by Newman–Keuls test).

0.001, one-way RM ANOVA followed by N-K test), and the intraburst frequency from 92 ± 9 to 70 ± 10 Hz ($n = 12$, $P < 0.001$, one-way RM ANOVA followed by N-K test). The effect of CNQX was not due to a change in the burst frequency, which was not affected by any of the blockers tested, consistent with previous results (Hayar et al. 2004a). Moreover, it could not be explained simply by a hyperpolarization of the membrane potential because we previously showed that membrane hyperpolarization actually increases the number of spikes per burst (Hayar et al. 2004a). In addition, the effect of CNQX could not be a consequence of gabazine-induced change in network activity because similar changes in bursting properties were produced when CNQX was applied together with APV and gabazine (see above). Therefore excitatory synaptic input that is mediated by non-NMDA ionotropic glutamate receptors may play a significant role in enhancing ET cell bursting by increasing the number of spikes per burst.

DISCUSSION

The present results demonstrate that ET cell bursting is controlled and coordinated by both excitatory and inhibitory synaptic inputs, which strengthen and weaken bursting, respectively, by modulating the number of spikes per burst. We recently showed that ET cells respond to continuous current injection by a change in burst frequency, membrane potential, and the number of spikes per burst, without a significant change in overall firing frequency (Hayar et al. 2004a). The average firing frequency (number of spikes/s) did not change significantly as the membrane potential was varied from -52 to -60 mV (Hayar et al. 2004a). Recent experiments and theoretical studies have provided accumulating evidence for the idea that the spike timing within bursts is regulated in such a way as to provide efficient and reliable information transmission between neurons (Derjean et al. 2003; Fanselow et al. 2001). Therefore neurotransmitters and neuromodulators, although demonstrably affecting the overall activity of oscillatory neural networks (Ruskin et al. 1999), also appear to play an important role in the regulation of the envelope and temporal structure of bursts.

In the glomerular layer, the rhythmic spike bursting was found exclusively in ET neurons and is an intrinsic property of these cells because their bursting persists during blockade of fast synaptic transmission as in subicular pyramidal neurons (Mason 1993). However, bursting in ET cells could also be facilitated by excitatory input as in midbrain dopaminergic neurons (Mereu et al. 1997). Several studies have suggested that bursts may form a particularly powerful signal within cortical networks. Moreover, bursting cells are in an ideal position to act as synchronizers of dynamically coupled neuronal networks. With bursts of action potentials the probability of synaptic failure is reduced and synaptic efficacy is enhanced. In particular, coincident bursts of action potentials invading the presynaptic terminals could be the most powerful stimulus to excite a postsynaptic neuron (for review, see Lisman 1997). Neurotransmitter release during a burst discharge can lead to an amplification mechanism even in synapses that undergo use-dependent depression (Williams and Stuart 1999). The temporal summation of excitatory postsynaptic potentials near the firing threshold could activate voltage-dependent channels and increase the likelihood of action potential initiation in the postsynaptic neuron in response to a presynaptic burst firing.

Because of their bursting properties, ET cells may amplify sensory input by converting a single olfactory nerve volley into an all-or none burst (Hayar et al. 2004a). However, the strength of the burst or the number of spikes in a burst may be finely tuned to the intensity of olfactory nerve input, which may be proportional to odor concentration. In addition, the burst strength may be dampened by inhibitory feedback from PG cells. Such fine-tuning, combined with entrainment of the ensemble of ET cells of the same glomerulus to a common excitatory input (Hayar et al. 2004a, 2005), may function to encode sensory input into a pattern of dynamic bursting code that reflects odor identity and concentration. Because ET cell axons were found to project to the anterior olfactory nucleus, the rostral-most component of olfactory cortex (Puche et al. 2005), ET cells may directly transmit odor information to cortical circuits.

REFERENCES

Derjean D, Bertrand S, Le Masson G, Landry M, Morisset V, Nagy F. Dynamic balance of metabotropic inputs causes dorsal horn neurons to switch functional states. *Nat Neurosci* 6: 274–281, 2003.

Fanselow EE, Sameshima K, Baccala LA, Nicolelis MA. Thalamic bursting in rats during different awake behavioral states. *Proc Natl Acad Sci USA* 98: 15330–15335, 2001.

Getchell TV, Shepherd GM. Synaptic actions on mitral and tufted cells elicited by olfactory nerve volleys in the rabbit. *J Physiol* 251: 497–522, 1975.

Hayar A, Karnup S, Ennis M, Shipley MT. External tufted cells: a major excitatory element that coordinates glomerular activity. *J Neurosci* 24: 6676–6685, 2004b.

Hayar A, Karnup S, Shipley MT, Ennis M. Olfactory bulb glomeruli: external tufted cells intrinsically burst at theta frequency and are entrained by patterned olfactory input. *J Neurosci* 24: 1190–1199, 2004a.

Hayar A, Shipley MT, Ennis M. Olfactory bulb external tufted cells are synchronized by multiple intraglomerular mechanisms. *J Neurosci* 25: 8197–8208, 2005.

Kauer JS. Olfactory processing: a time and place for everything. *Curr Biol* 8: R282–R283, 1998.

Kay LM, Laurent G. Odor- and context-dependent modulation of mitral cell activity in behaving rats. *Nat Neurosci* 2: 1003–1009, 1999.

Laurent G, Wehr M, Davidowitz H. Temporal representations of odors in an olfactory network. *J Neurosci* 16: 3837–3847, 1996.

Lisman JE. Bursts as a unit of neural information: making unreliable synapses reliable. *Trends Neurosci* 20: 38–43, 1997.

Llinas RR. The intrinsic electrophysiological properties of mammalian central neurons: insights into central nervous system function. *Science* 242: 1654–1664, 1988.

Mason A. Electrophysiology and burst-firing of rat subicular pyramidal neurons in vitro: a comparison with area CA1. *Brain Res* 600: 174–178, 1993.

Mereu G, Lilliu V, Casula A, Vargiu PF, Diana M, Musa A, Gessa GL. Spontaneous bursting activity of dopaminergic neurons in midbrain slices from immature rats: role of N-methyl-D-aspartate receptors. *Neuroscience* 77: 1029–1036, 1997.

Puche AC, Parrish-Aungst S, Valentino K, Shipley MT. External tufted cells: instant messaging to the cortex? *Soc Neurosci Abstr* 740.14, 2005.

Ruskin DN, Bergstrom DA, Walters JR. Multisecond oscillations in firing rate in the globus pallidus: synergistic modulation by D1 and D2 dopamine receptors. *J Pharmacol Exp Ther* 290: 1493–1501, 1999.

Wehr M, Laurent G. Odour encoding by temporal sequences of firing in oscillating neural assemblies. *Nature* 384: 162–166, 1996.

Wellis DP, Scott JW. Intracellular responses of identified rat olfactory bulb interneurons to electrical and odor stimulation. *J Neurophysiol* 64: 932–947, 1990.