
Characterization of the Synaptic Properties of Olfactory Bulb Projections

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Abstract

The olfactory bulb directly projects to several diverse telencephalic structures, but, to date, few studies have investigated the physiological characteristics of most of these areas. As an initial step towards understanding the odor processing functions of these secondary olfactory structures, we recorded evoked field potentials in response to lateral olfactory tract stimulation *in vivo* in urethane-anesthetized Sprague–Dawley rats in the following brain structures: anterior olfactory nucleus, ventral and dorsal tenia tecta, olfactory tubercle, anterior and posterior piriform cortex, the anterior cortical nucleus of the amygdala, and lateral entorhinal cortex. Using paired-pulse stimulation with interpulse intervals of 25–1000 ms, we observed facilitation of the response to the second pulse in every structure examined, although the degree of facilitation varied among the target structures. Additionally, pulse train stimulation at three different frequencies (40, 10 and 2 Hz) produced facilitation of evoked field potentials that also varied among target structures. We discuss the potential utility of such short-term facilitation in olfactory processing.

Key words: olfactory cortex, olfaction, paired-pulse facilitation, rat, short-term plasticity

Introduction

Secondary olfactory structures are those areas of the brain that receive direct projections from the olfactory bulb (for review see Price, 1987; Cleland and Linster, 2003). These areas include the anterior olfactory nucleus (AON), ventral tenia tecta (VTT), dorsal tenia tecta (DTT; or anterior hippocampal continuation), olfactory tubercle (OT), anterior and posterior piriform cortices (aPC, pPC), anterior cortical nucleus of the amygdala (ACo), and lateral entorhinal cortex (LEC). Among these diverse structures, only the piriform cortex has been well described electrophysiologically, exhibiting paired-pulse facilitation (Richards, 1972; Haberly, 1973; Bower and Haberly, 1986), long-term potentiation of the response to lateral olfactory tract (LOT) stimulation after odor learning (Roman *et al.*, 1993), long term potentiation and depression between pyramidal neurons (Lebel *et al.*, 2001), and associative long-term potentiation between afferent and association fibers under certain modulatory conditions (Kanter and Haberly, 1993; Patil *et al.*, 1998). Relatively little is known about the synaptic physiology and plasticity properties of the other secondary olfactory structures, and their specific functions remain largely unknown. However, recent studies in humans, as well as physiological and behavioral experiments in nonhuman mammals, have begun to provide insight into

the roles that these structures may play in integrative olfactory processing (reviewed in Cleland and Linster, 2003).

We therefore examined the responses of a number of secondary olfactory structures to LOT stimulation *in vivo* in the rat. During stimulation of the LOT with paired pulses or with trains of twenty pulses at different interpulse intervals (IPIs), we observed facilitation of evoked field potentials (EFPs) in layer Ia of each of these structures. Although every structure demonstrated facilitation, the degree of facilitation varied among structures. We discuss the functional implications of this short-term synaptic plasticity with respect to the associative and computational tasks that these divergent olfactory structures may perform.

Methods

Experimental subjects

Adult female Sprague–Dawley rats (250–300 g) were anesthetized with urethane (1.5 g/kg intraperitoneal; Sigma Chemicals, St Louis, MO). Levels of anesthesia were monitored by respiration rate and foot withdrawal reflex and supplemented by further intraperitoneal injections, if necessary. Throughout surgery and recording, body temperature was maintained with heating pads. Anesthetized animals were placed in a stereotaxic apparatus (Narishige Scientific

Instruments, Tokyo, Japan) and the skull was exposed with a scalpel incision. Holes were drilled to expose the LOT and various secondary olfactory structures (see Table 1 for stereotaxic coordinates) (Paxinos and Watson, 1998). All procedures were performed under guidelines established by the Cornell University Institutional Animal Care and Use Committee and the National Institutes of Health.

Electrical stimulation: paired pulse experiments

Bipolar stimulating electrodes (100 μm stainless steel, Formvar-insulated) were placed in the LOT. Optimal placement was achieved by monitoring the size and shape of field potentials in the target secondary olfactory structure evoked by LOT stimulation (see Haberly, 1973 for details). Stimulation currents were delivered by a constant-current stimulus isolation unit (Model PSIU6) controlled by a Grass S88 stimulator (Grass-Telefactor, W. Warrick, RI). Paired pulses of 200–900 μA amplitude and 0.1 ms duration were delivered at interpulse intervals of 25, 50, 100, 250, 500 and 1000 ms for 10 trials each with 5 s between each trial. A final trial with an IPI of 25 ms was repeated 5 s after the final 1000 ms trial to ensure that no lasting changes in evoked response properties had occurred during the experiment. Sixteen rats were used in the paired pulse experiments.

Electrical stimulation: train experiments

The LOT was stimulated by 20-pulse trains at frequencies of 40, 10 and 2 Hz (i.e. with IPIs of 25, 100 and 500 ms, respectively) while recording from each of the structures listed in Table 1. Ten trials at each frequency were done during each experiment, and each trial was separated by 5 s. Experimental set-up and equipment and pulse parameters were otherwise identical to those in the paired pulse experiments. Recordings were performed in seven rats.

Recording

Evoked field potentials were recorded with 100 μm stainless steel recording electrodes in each of the structures listed in Table 1. Signals were bandpass filtered between 0.1 Hz and 3 kHz, amplified $\times 1000$ (P55 AC preamplifier, Grass-Telefactor), and sampled at 20 kHz. Stimulus control was performed with CED Power1401 hardware and Spike2 software (Cambridge Electronic Design, Cambridge, UK), as were data acquisition, display, and analysis. The recording electrodes were placed in layer Ia of the target structures in such a way as to observe a short latency (5–7 ms) response to stimulation of the LOT (Figure 1). The EFP observed in layer Ia of olfactory cortical structures in response to LOT stimulation exhibits an early negative peak (A1, see Haberly, 1973, 1998) thought to be generated by a monosynaptic excitatory postsynaptic potential. Current source density analysis has shown that current associated with the generation of a monosynaptic EPSP in pyramidal cells underlies the falling phase and initial peak of this evoked field

Table 1 Stereotaxic coordinates and abbreviations of recording sites

Structure	Abbreviation	Coordinates
Lateral olfactory tract	LOT	+3.7AP; 3.4ML; 6.5DV
Anterior olfactory nucleus	AON	+5.2AP; 1.8ML; 4.2DV
Ventral tenia tecta	VTT	+3.7AP; 1.0ML; 7.6DV
Dorsal tenia tecta	DTT	+3.7AP; 1.5ML, 12°; 6.4DV
Olfactory tubercle (cortical) ^a	OT	+1.2AP; 3.0ML; 7.5–8DV
Olfactory tubercle (striatal) ^a	OT	+1.2AP; 1ML; 8.8DV
Anterior piriform cortex	aPC	+1.2AP; 3.0ML, 14°; 7.5DV
Posterior piriform cortex	pPC	-2.12AP; 2.0ML, 32°; 8.5DV
Anterior cortical nucleus of the amygdala	ACo	-2.12AP; 3.4ML; 9.2DV
Lateral entorhinal cortex	LEC	-5.2AP; 4.0ML, 21°; 10DV

AP: anterior–posterior axis; ML: medial–lateral axis; DV: dorsal–ventral axis; coordinates from Paxinos and Watson (1998).

^aRecordings were made from two different locations in the olfactory tubercle, based on anatomical differences (Millhouse and Heimer, 1984; Cleland and Linster, 2003). There were no significant differences between responses from the two recording sites, so data were combined for analysis and are collectively referred to as the olfactory tubercle in the text.

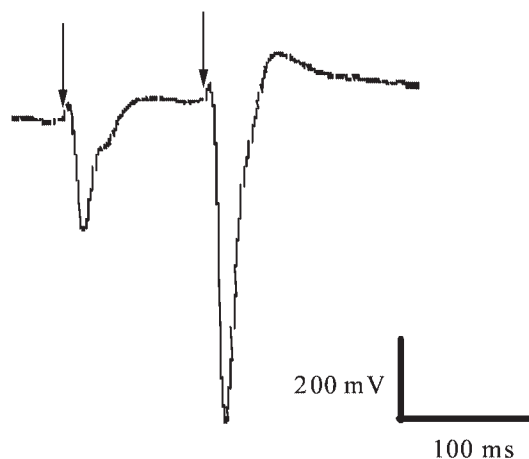


Figure 1 Evoked field potentials from the posterior piriform cortex. Evoked field potential response of posterior piriform cortex to paired-pulse stimulation of the lateral olfactory tract (600 μA for 0.1 ms, IPI = 100 ms). Arrows indicate the times at which the LOT was stimulated. The average of 10 response traces is depicted. Note the dramatic increase in amplitude of the second EFP due to facilitation. The EFPs recorded from each structure were similar in appearance.

potential component (Ketchum and Haberly, 1993a,b); as a consequence, the efficacy of the afferent fiber synapse was assessed by measuring the maximally negative onset slope of this EFP (see also Kapur and Haberly, 1998). Measurement of EFP onset slope rather than peak amplitude also reduces error due to baseline changes and noise that are inherent in the latter method.

Histology

Recording and stimulation sites were marked by passing current through the electrodes with a 9V battery to deposit iron in the tissue. Rats were sacrificed with transcardial perfusion of 0.9% saline followed by 4% potassium ferrocyanide (Sigma-Aldrich, St. Louis, MO) in 10% buffered formalin (Fisher Chemicals, Fair Lawn, NJ). Brains were stored in 10% formalin with 20% sucrose for at least 24 h, followed by sectioning on cryostat and staining with Neutral red (Allied Chemicals, New York, NY) in order to visualize

the locations of the recording and stimulating electrodes. Only recordings for which electrode locations were clearly identified in the target structure were included in the data analysis. Figure 2 shows an example of histological data and summarizes the locations of all recording sites.

Data analysis

For paired pulse data, the maximum slope of the second EFP (EFP_2) was compared with that of the first EFP (EFP_1) using a two-tailed paired *t*-test ($\alpha = 0.05$) for each structure

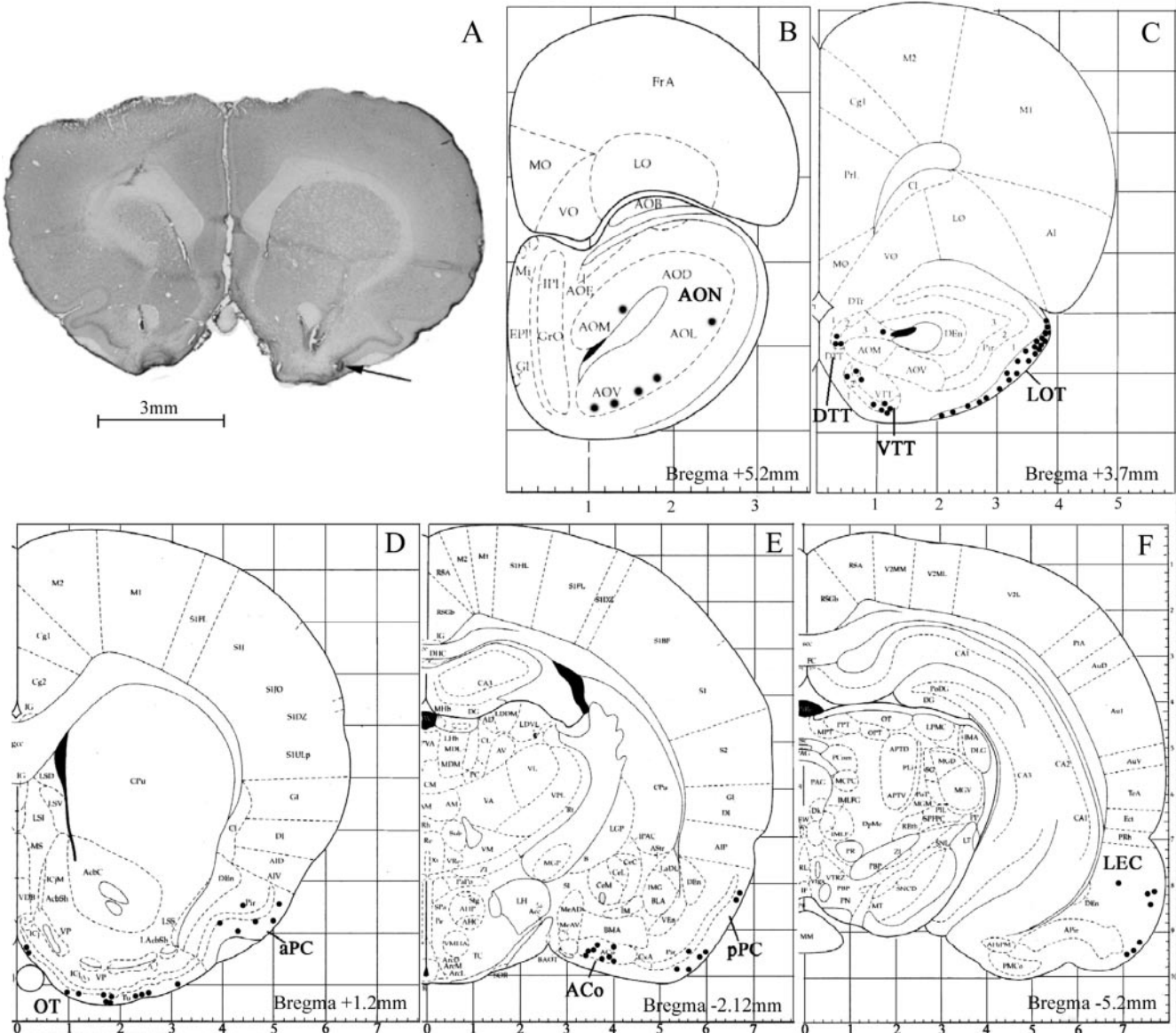


Figure 2 Localization of the recording electrodes. All recording sites were verified histologically using coronal sections. (A) A coronal section at the level of the OT (1.2 mm anterior to bregma) shows the marked recording site indicated by the arrow. (B–F) Schematic drawings at five different locations relative to bregma summarize the stimulation and recordings sites used in the experiments presented here (modified from Paxinos and Watson, 1998). (B) AON (5.2 mm anterior to bregma). (C) VTT, DTT and LOT (3.7 mm anterior to bregma). (D) aPC and OT (1.2 mm anterior to bregma). (E) pPC and ACo (2.12 mm posterior to bregma). (F) LEC (5.2 mm posterior to bregma). For all drawings, each grid box scales to 1 mm × 1 mm. Reprinted from Paxinos and Watson (1998), with permission from Elsevier.

and IPI. For graphing purposes, the EFP_2 was normalized by calculating the ratio $EFP_2:EFP_1$; these paired pulse ratios (PPRs), with standard error, are depicted in the figures. An analysis of variance of the paired pulse ratios was performed to assess the overall differences between facilitation ratios with respect to IPI and structure; *post hoc* tests (Tukey honestly significant difference; HSD) were subsequently used to ascertain significant differences attributable to differences in IPI or neural structure.

For train stimulation data, analyses of variance were performed at each IPI to compare EFP onset slopes in response to each of the 20 pulses in a train; *post hoc* tests (Tukey HSD) were then performed to determine which of the responses (EFP_{2-20}) differed significantly from the first (EFP_1). For ease of visualization, the mean maximum slope of each EFP was normalized by dividing by the mean slope of EFP_1 ; these facilitation ratios (FRs) are depicted on graphs, with standard error.

When grouped data are presented as $X \pm Y$, Y represents SEM.

Results

Stimulation of the LOT generated an evoked field potential in each of the secondary olfactory structures examined. The EFP constituted a negative peak followed by a positive component (Figure 1) in all structures, as has been previously described for piriform cortex (Haberly, 1973; Linster *et al.*, 1999); the early negative peak was measured in this study, as this corresponds to the monosynaptic input from the olfactory bulb (Ketchum and Haberly, 1993a, Linster *et al.*, 1999). Also indicative of a monosynaptic response, the latency between stimulation of the LOT and EFP peak amplitude occurred between 4 and 10 ms for every structure, depending on the distance between recording and stimulation sites (for instance, the AON had the shortest latency and the LEC had the longest latency to peak). Paired pulse stimulation evoked significant facilitation of the second EFP in all secondary olfactory structures examined in this study, although the degree of this facilitation varied among structures. Facilitation was also observed in all structures during train stimulation; again, the degree of this facilitation varied among structures.

Paired-pulse stimulation

In the AON, the maximum slope of the second evoked field potential in each pair was significantly greater ($P < 0.001$) than the maximum slope of the first EFP at IPIs ranging from 25 to 500 ms (Figure 3, AON). Peak facilitation in this structure occurred at an IPI of 50 ms ($PPR = 1.63 \pm 0.04$).

An evoked field potential was also recorded in the VTT following LOT stimulation. The maximum slope of EFP_2 was significantly greater than the slope of EFP_1 at IPIs ranging from 25 to 500 ms ($P < 0.001$ for all IPIs; Figure 3, VTT). Maximum facilitation occurred at an IPI of 50 ms

($PPR = 1.77 \pm 0.03$); the degree of facilitation declined as the IPI increased.

In contrast to the AON and the VTT, the DTT exhibited paired pulse facilitation at IPIs between 25 ms and 100 ms only ($P < 0.001$; Figure 3, DTT). Peak facilitation occurred at the smallest IPI of 25 ms ($PPR = 1.61 \pm 0.04$).

The OT exhibited paired pulse facilitation at every IPI tested ($P < 0.02$ for all IPIs; Figure 3, OT). The greatest facilitation was at an IPI of 50 ms ($PPR = 1.58 \pm 0.06$).

We recorded from the piriform cortex in two different regions, the aPC and the pPC, since earlier studies have suggested that there is a functional dissociation between these regions (Chabaud *et al.*, 1999). In the aPC, paired pulse stimulation evoked facilitation of the second EFP at IPIs of 25–250 ms ($P < 0.0001$ for all IPIs; Figure 3, aPC); peak facilitation occurred with a 25 ms IPI ($PPR = 2.07 \pm 0.7$). In contrast, we saw significant facilitation of the second EFP in the pPC at every IPI tested ($P < 0.01$ for all IPIs; Figure 3, pPC); peak facilitation also occurred at 25 ms IPI in this structure ($PPR = 1.98 \pm 0.06$).

The ACo exhibited facilitation with paired-pulse stimulation at IPIs between 25 ms and 500 ms ($P < 0.002$; Figure 3, ACo). Peak facilitation occurred with a 50 ms IPI ($PPR = 1.69 \pm 0.07$).

The LEC exhibited the least facilitation of all the secondary olfactory structures. Facilitation was significant at IPIs between 25 ms and 250 ms ($P < 0.004$; Figure 3, LEC), but the maximum paired pulse ratio of 1.26 ± 0.04 (at IPI = 50 ms) was the smallest peak facilitation seen in any structure.

Summary

Paired-pulse facilitation was observed in all structures tested, albeit to varying degrees. Maximal facilitation always occurred at the two smallest IPIs (25 or 50 ms in all structures tested); and facilitation was observed for a range of IPIs in all structures. The main difference observed between structures was the degree of facilitation, as can be seen in Figure 3. An analysis of variance comparing the paired pulse ratios at all IPIs and in all structures revealed significant differences between IPIs [$F(5, 3115) = 300.6$; $P < 0.001$] and between structures [$F(8, 3115) = 50.6$; $P < 0.001$]; in addition, a significant interaction between IPI and structure was noted [$F(40, 3115) = 9.7$; $P < 0.001$]. *Post hoc* tests (Tukey HSD) demonstrated significant differences ($P < 0.001$) between all individual IPIs with the exception of 500 and 1000 ms ($P > 0.5$). *Post hoc* tests also showed significant differences of paired pulse ratio between some, but not all, structures; the results from these *post hoc* tests are summarized in Table 2. Interestingly, facilitation in the aPC and pPC were significantly different from that in all other structures ($P < 0.001$), but not from each other; similarly, paired pulse ratios in the LEC were significantly different from those in all other structures (Table 2).

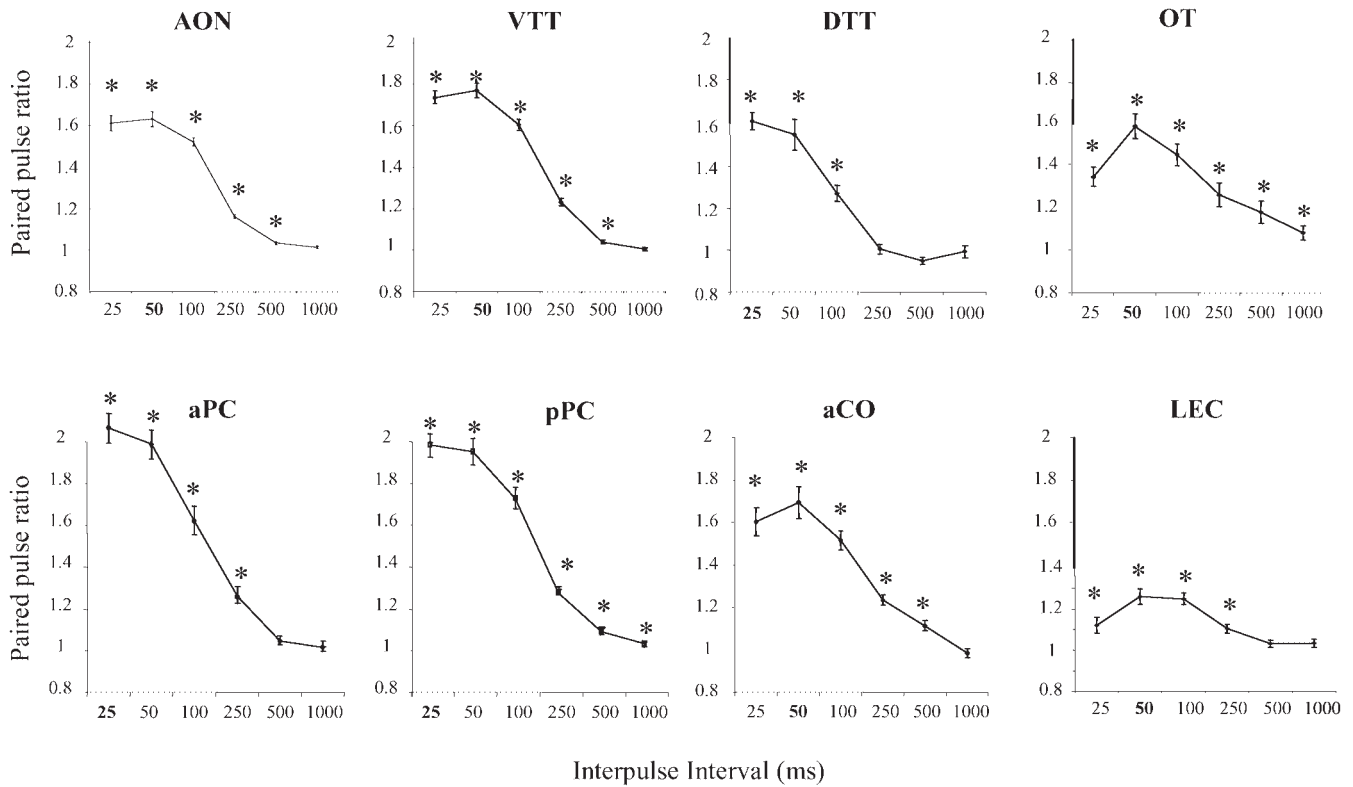


Figure 3 Responses to paired-pulse stimulation. Paired-pulse ratios of evoked field potential (EFP) amplitude in response to LOT stimulation are depicted as a function of interpulse interval (IPI). For each IPI and structure, the slope of the response to the second pulse was divided by that of the response to the first pulse. All values are depicted as mean \pm SEM. The IPI for which the maximal paired pulse ratio was obtained in each structure is indicated in bold letters. AON: accessory olfactory nucleus; VTT: ventral tenia tecta; DTT: dorsal tenia tecta or anterior hippocampal continuation; OT: olfactory tubercle; aPC: anterior piriform cortex; pPC: posterior piriform cortex; ACo: anterior cortical nucleus of the amygdala; LEC: lateral entorhinal cortex.

Table 2 Summary of statistical comparison between all structures

	AON	VTT	DTT	OT	aPC	pPC	ACo	LEC
AON	–		*		*	*		*
VTT		–	*	*	*	*		*
DTT			–	*	*	*	*	*
OT				–	*	*		*
aPC					–		*	*
pPC						–	*	*
ACo							–	*
LEC								–

Asterisks indicate pairs of structures for which significant differences in paired pulse ratio were observed.

All of our experimental series began with stimulation at a 25 ms IPI. Five seconds after the final 1000 ms IPI trial, we repeated the 25 ms IPI stimulus pattern. In all structures, we saw facilitation consistent with that evoked by the first 25 ms IPI stimulation, and the majority of structures were statistically stationary in absolute maximum EFP slope and paired pulse ratio over the course of the experiment (data

not shown). In the AON, ACo and LEC, however, we saw slight but significant increases in paired pulse ratios in the second 25 ms experiment compared with the first. In the AON, the second 25 ms experiment evoked a paired pulse ratio of 1.74 ± 0.067 , compared with 1.60 ± 0.056 in the first experiment ($P < 0.05$). In the ACo, the paired pulse ratios were 1.74 ± 0.067 and 1.60 ± 0.056 ($P < 0.05$) in the second and first 25 ms experiments, respectively. Finally, the paired pulse ratio in the LEC of the second 25 ms experiment was 1.37 ± 0.048 , in contrast to 1.12 ± 0.038 in the first 25 ms experiment ($P < 0.005$). These differences were not large, but may reflect some long-term facilitation, which could have slightly influenced our results in these structures.

Train stimulation

In response to pulse-train stimulation of the LOT, facilitation of the synaptic responses was observed in all structures in response to 40 Hz and 10 Hz stimulation, and in response to 2 Hz stimulation in five structures (AON, OT, pPC, ACo and LEC), as indicated by a significant effect of pulse number using ANOVA and a significant difference in *post hoc* statistics between the response to the first pulse and that to subsequent pulses. In all cases, maximum facilitation occurred after the second pulse (typically between the third

and sixth pulse inclusive), indicating that paired-pulse stimulation does not necessarily evoke the highest possible facilitation. In addition, facilitation typically declined after maximal facilitation had been reached, although responses remained significantly facilitated throughout the duration of the train. Examples of pulse-train stimulation responses in two structures with different degrees of facilitation (VTT and pPC) are depicted in Figure 4; Table 3 summarizes the results from the pulse-train experiments obtained in all structures.

Discussion

Among secondary olfactory structures, the piriform cortex has been the most extensively studied (Haberly, 1973; Schwob *et al.*, 1984; Bower and Haberly, 1986; Hasselmo and Bower, 1990; Ketchum and Haberly, 1993a,b; Wilson, 1997, 1998, 2000; Linster *et al.*, 1999; Illig and Haberly,

2003), including studies of field potential responses to paired pulse stimulation of the LOT (Richards, 1972; Haberly, 1973; Bower and Haberly, 1986). The present work extends these studies into the multiple secondary olfactory structures receiving direct, divergent input from olfactory bulb mitral cells. Since Haberly and Bower (1989) first proposed that the piriform cortex may mediate associative memory function in the olfactory system, several subsequent modeling and electrophysiological studies have investigated the conditions under which such functions could be implemented by the piriform cortical architecture (Hasselmo *et al.*, 1990; Hasselmo and Barkai, 1995; Wilson, 1997; Haberly, 1998, 2001; Saar *et al.*, 1998, 1999; Johnson *et al.*, 2000; Barkai and Saar, 2001; Linster and Hasselmo, 2001). In addition to its suggestive architecture, the connections of piriform cortex to multimodal processing and limbic areas such as the entorhinal cortex, hippocampus, and amygdala support the

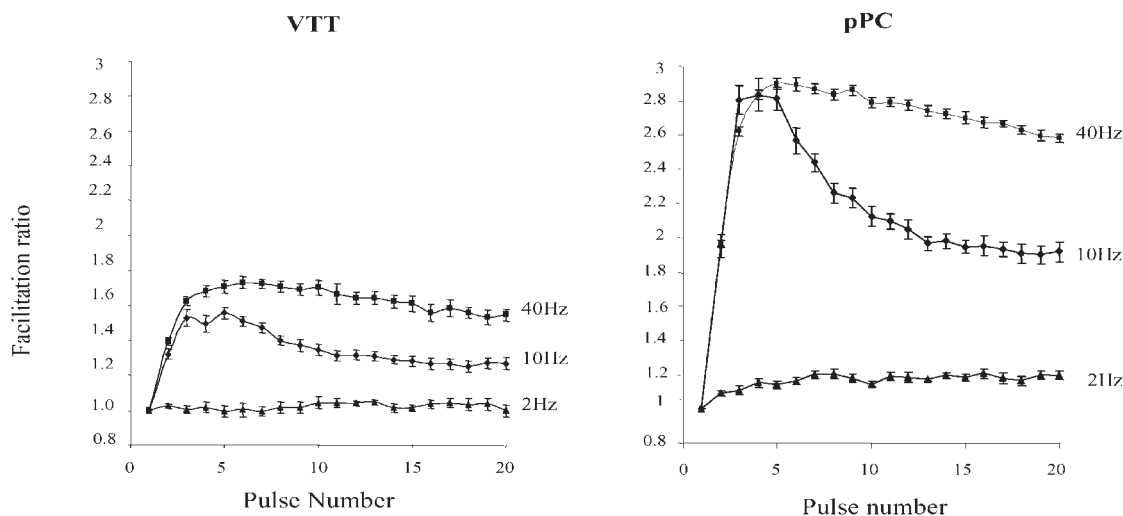


Figure 4 Responses to train stimulation. Facilitation ratios (FR) for each EFP in two structures structure following train stimulation at three different stimulation frequencies (40, 10 and 2 Hz) are shown as a function of the pulse number. At each frequency, the slopes of the responses were normalized to that of the first response. VTT: ventral tenia tecta; pPC: posterior piriform cortex.

Table 3 Summary of pulse-train stimulation results

	40 Hz train		10 Hz train		2 Hz train	
	Maximum facilitation	Maximum at	Maximum facilitation	Maximum at	Maximum facilitation	Maximum at
AON	1.8 ± 0.12*	EFP ₄	2.3 ± 0.2*	EFP ₅	1.2 ± 0.05*	EFP ₆
VTT	1.55 ± 0.03*	EFP ₅	1.73 ± 0.03*	EFP ₆	NS	—
DTT	2.4 ± 0.13*	EFP ₅	1.96 ± 0.15*	EFP ₆	NS	—
OT	2.19 ± 0.07*	EFP ₃	1.54 ± 0.05*	EFP ₄	1.09 ± 0.02*	EFP ₂
aPC	2.19 ± 0.07*	EFP ₄	2.14 ± 0.03*	EFP ₅	NS	—
pPC	2.83 ± 0.09*	EFP ₄	2.9 ± 0.03*	EFP ₅	1.2 ± 0.02*	EFP ₇
ACo	1.61 ± 0.09*	EFP ₄	1.67 ± 0.09*	EFP ₆	1.17 ± 0.03*	EFP ₈
LEC	1.72 ± 0.08*	EFP ₆	1.9 ± 0.03*	EFP ₅	1.12 ± 0.03*	EFP ₆

*Significant facilitation of the EFP ($P < 0.05$). NS = no significant facilitation of the EFP.

assertion that the piriform cortex could support associative memory functions (Haberly, 2001).

Of course, other secondary olfactory structures are also likely to be involved in shaping the construction of olfactory associative memories. For example, Haberly (2001) has suggested that the AON (which he terms the anterior olfactory cortex to emphasize its cortical architecture, and which projects centrally via the layer Ib fiber system) constructs 'gestalts', or holistic representations, of olfactory information, rather than parsing the odorant into its individual components. In addition, he suggested a role in retrograde memory recall for medial olfactory cortices such as the tenia tecta and dorsal peduncular cortex, which project via the layer II-deep Ib fiber system. The increasing evidence for such integrative hypotheses underscores the potential importance of these diverse secondary olfactory structures in the discrimination and learning of odor stimuli, and in the production of appropriate behavioral responses. The present study characterizes the responses of these diverse secondary olfactory structures to pulse train inputs from olfactory bulb mitral cells, as a first step to understanding the utility of the divergence and subsequent reintegration of bulbo-cortical projections for olfactory processing, learning, and memory.

Excitatory synapses formed by mitral cell terminals within secondary olfactory structures all showed short-term facilitation in response to paired pulse and pulse train stimulation; this increase in evoked field potential amplitude varied between 30–100% depending on the secondary structure in question and the interpulse interval. Maximum facilitation in all structures occurred at IPIs of 25–50 ms; significant differences between the responses at these two IPIs were found only in the OT and LEC, which were maximally facilitated at an IPI of 50 ms. Hence, maximal facilitation in these secondary structures generally occurred in response to spike frequencies of 20 Hz and above, which corresponds with the spectral peak of external plexiform layer/granule cell layer oscillations evoked by tetanic stimulation of glomerular inputs in bulb slices (Friedman and Strowbridge, 2003), as well as with the intrinsic oscillatory frequencies of individual mitral cells (Chen and Shepherd, 1997). In addition, this sensitivity to frequencies above 20 Hz may be related to the frequencies of beta/gamma band oscillations (12–80 Hz) measured in the olfactory bulb and piriform cortex that reflect the synchronization of driver potentials and hence the frequency of spiking (Adrian, 1950; Eeckman and Freeman, 1990; Kay and Freeman, 1998). In particular, odor-induced fast wave field potentials in the piriform cortex peak at ~20 Hz (Zibrowski and Vanderwolf, 1997; Chabaud *et al.*, 1999).

The time course of facilitation was relatively consistent across structures, although the degree of facilitation varied, as did the differences between peak and steady-state facilitation ratios. This consistency suggests that the underlying determinants of time course may be presynaptically defined,

i.e. the determinants may be properties of the mitral cells that synapse onto pyramidal cells in these diverse secondary structures. This would be consistent with the 'synaptic homogeneity principle' suggested by Gupta *et al.* (2000), with which those authors described GABAergic interneurons that form synapses that differ in strength, but exhibit similar temporal dynamics, onto cells of the same class.

While the mechanisms underlying short-term changes in synaptic efficacy have been extensively studied, the functional role these changes play is not well understood (Fortune and Rose, 2001). Nevertheless, some recent proposals regarding the computational utility of short-term plasticity may be applicable to the olfactory system. First, facilitation of cortical responsiveness to paired or bursting presynaptic action potentials may increase the distinction between single and paired spikes. Mitral cells, the axons of which constitute the afferent LOT, are bistable neurons with an upstate that is perithreshold to action potential firing (Heyward *et al.*, 2001); hence, many mitral cells exhibit spontaneous activity (Wellis *et al.*, 1989; Chen and Shepherd, 1997). In response to odor stimulation, those mitral cells that are initially excited by the odorant tend to fire bursts of spikes (Wellis *et al.*, 1989), and individual mitral cells respond to prolonged depolarization by firing repetitive bursts of action potentials (Chen and Shepherd, 1997), potentially serving to differentiate stimulus-evoked responses from spontaneous activity. In such noisy systems, single spikes may be de-emphasized, or even ignored, by the postsynaptic cell (Lisman, 1997); facilitation of the response to paired or bursting presynaptic action potentials may filter the signal so as to emphasize genuine stimulus-driven responses over spontaneous single spikes.

Second, central synapses can also be unreliable in the sense that release of transmitter is probabilistic, hence a single presynaptic spike can fail to evoke a response in the postsynaptic cell (Stevens and Wang, 1995; Lisman, 1997). Facilitating synapses often are unreliable in this sense, exhibiting relatively low probabilities of release and high transmission failure rates (Gupta *et al.*, 2000; Maruki *et al.*, 2001). Indeed, the facilitating synaptic terminals of LOT afferents in the PC have low synaptic vesicle packing densities, which could indicate a low probability of neurotransmitter release (Bower and Haberly, 1986).

We did not see depression in any secondary olfactory structure with either stimulation protocol (aside from a slight and inconsistent depression observed in the dorsal tenia tecta at low stimulation frequency), a result consistent with paired-pulse studies in the piriform cortex, both in slice (Bower and Haberly, 1986) and *in vivo* (Haberly, 1973). This does not necessarily mean that these synapses are incapable of depressing, as Richards (1972) did see slight depression in slices of guinea-pig piriform cortex at IPIs > 300 ms and after low frequency conditioning trains. Additionally, Hasselmo and Bower (1990) found that train stimulation of layer Ia in aPC slices caused a decrease in the average EFP

amplitude in layer II pyramidal cells 50 s after train cessation compared with before the train stimulation, although the LOT synapses onto these cells still facilitated in response to paired pulse stimulation. Furthermore, a secondary depression process could account for the decline in facilitation ratio observed over the course of pulse train stimulation, suggesting a mixture of facilitation and depression in the population response. Such combinations of facilitation and depression processes have been described at other cortical synapses (Varela *et al.*, 1997; Hempel *et al.*, 2000).

In summary, we have demonstrated that the LOT synapses in multiple secondary olfactory structures are facilitating, with the synapses in different structures exhibiting consistent commonalities and differences. The facilitation properties of these synapses suggest interesting possibilities and constraints for how synaptic plasticity is involved in the computational processing of odor stimuli at higher levels in the olfactory system.

Acknowledgements

This work was supported by NIH Training Grant T32 GM07469 (AMM), the Alfred P. Sloan Foundation (CL), and NIDCD (CL). We would like to thank Bruce R. Johnson and Sarah W. Newman for helpful comments on a previous version of the manuscript.

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Accepted January 26, 2004