MECHANISMS AND FUNCTION OF NEURAL SYNCHRONIZATION IN AN INSECT OLFACTORY SYSTEM

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In memory of my father, Norman MacLeod (1943-1997)

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Abstract

One of the fundamental questions in modern integrative neurobiology relates to the encoding of sensory information by populations of neurons, and to the significance of this activity for perception, learning, memory and behavior. Synchronization of activity across a population of neurons has been observed many times over, but has never been demonstrated to be a necessary component of this coding process. Neural synchronization has been found in many brain areas in animals across several phyla, from molluscs to mammals. Studies in mammals have correlated the degree of neural synchronization with specific behavioral or cognitive states, such as sensorimotor tasks, segmentation and binocular rivalry suggesting a functional link. In the locust olfactory system, oscillatory synchronization is a prominent feature of the odor-evoked neural activity. Stimulation of the antenna by odors evokes synchronized firing in dynamic and odor-specific ensembles of the projection neurons of the antennal lobe, the principal neurons of the first-order olfactory relay in insects. The coherent activity of these projection neurons underlies an odor-evoked oscillatory field potential which can be recorded in the mushroom body, the second-order olfactory relay to which they project.

In this dissertation, we investigated two important questions raised by these findings: how are such stimulus-evoked synchronous ensembles generated, and what is their functional significance? To address these questions, we performed electrophysiological experiments and recorded odor responses from neurons of the antennal lobes and mushroom bodies of locusts, *in vivo* and using natural odor stimulation in an unanesthetized, semi-intact preparation.

We demonstrated the critical mechanism involved in neural synchronization of the antennal lobe neurons. The synchronization of the projection neurons relies critically on fast GABA (γ-aminobutyric acid) -mediated inhibition from the local interneurons.

Projection neuron synchronization could be selectively blocked by local injection of the GABA receptor antagonist, picrotoxin. Picrotoxin spared the odor-specific, slow modulation of individual projection neuron responses, but desynchronized the firing of the odor-activated projection neuron assemblies. The oscillatory activity of the local interneurons was also blocked by picrotoxin, which indicates that such activity depends on network synaptic dynamics. We also showed that the mushroom body networks are capable of generating oscillatory behavior of a similar frequency as that of its projection neuron inputs, and that they may thus be "tuned" to accept synchronized, oscillatory inputs of that frequency range.

Our understanding of this mechanism, in turn, made possible the functional investigation of neural synchronization by selective disruption of projection neuron synchronization. We studied a population of neurons downstream from the antennal lobe projection neurons, the extrinsic neurons of the β-lobe of the mushroom body (βLNs). These βLNs were chosen for investigation because they were found to be odor-responsive and because their position in the olfactory pathway makes them a suitable "read-out" of population activity in the antennal lobe. We characterized βLN odor responses before and after selective disruption of the synchronization of the projection neuron ensembles with local picrotoxin injection into the antennal lobe. We showed that the tuning of these βLN responses was altered by PN desynchronization by changing existing responses and inducing new responses. This alteration in tuning resulted in a significant loss of odor specificity in individual βLN responses, an effect that *never* occurred in the responses of individual, desynchronized projection neurons. We thus propose that neural synchronization is indeed important for information processing in the brain: it serves, at least in part, as a temporal substrate for the transmission of information that is contained *across* co-activated neurons (relational code) early in the pathway.

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Chapter 1: Background and significance.

I. Introduction

In this dissertation, we investigated the generation and functional significance of oscillatory neural synchronization in the olfactory system of the locust, *Schistocerca americana*. The results indicated that (1) the critical mechanism underlying neural synchronization in the antennal lobe involves a fast, GABA (γ-aminobutyric acid) -mediated inhibition and (2) neural synchronization of antennal lobe inputs is required for appropriate odor tuning of higher-order olfactory neurons found two synapses dowstream. The discovery that the mechanism of synchronization was independent of other activity shaping mechanisms (e.g., those that mediate odor tuning) in antennal lobe projection neurons provided the means to test, physiologically and *in vivo*, the functional significance of neural synchronization in a way not yet possible in other systems.

To provide a framework for these results, we review in Chapter 1 the literature on olfaction and neural coding, olfactory system structure and function in insects and vertebrates, and neural synchronization in non-olfactory systems.

We briefly outline the general problem of olfaction in Chapter 1, Section II. What is the nature of an olfactory stimulus? What does the behavior of animals suggest about olfactory information processing in the brain? What types of strategies might olfactory nervous systems use to encode this olfactory information? The answers to these questions provide a framework for understanding the physiological experiments reported in this thesis.

The design of olfactory nervous systems, e.g., local circuit architecture, anatomical and neuronal morphologies, is remarkably similar across many animal phyla. The study of the functional principles in one system, then, may provide insight for function in the

others, as well as provide information on general solutions for olfactory coding. We review here the structure (Section III) and function (Section IV) of the olfactory systems of insects and vertebrates, including several explicit models of olfactory function. For space considerations, the discussion is limited to these two systems, although the olfactory systems of crustacea, molluscs, and other invertebrates might also have been included. Also in Section IV, we discuss the specific finding of oscillatory neural synchronization, another common feature, in the locust and vertebrate olfactory systems.

Synchronization has also been described in the visual, auditory, and somatomotor cortical and subcortical areas and in the hippocampus. Studies in mammals, for example, have correlated the degree of neural synchronization with specific behavioral or cognitive tasks, such as segmentation, rivalry, and sensorimotor tasks, suggesting a functional link. We review a selection of these results, and the mechanisms proposed to underlie synchronization in these systems (Section V).

II. Olfactory Coding.

The problem of olfaction.

Olfaction involves many processes: chemical detection, discrimination of chemical components and complex blends, recognition of behaviorally relevant signals, association of an "odor object" with other events (e.g., rewards), and memory retrieval. The neural correlates of these functions probably depend on many overlapping mechanisms. Animals must use olfaction for a variety of behaviors necessary for survival, such as finding food, predator avoidance, mate selection and sexual behavior, navigation, and kin recognition.

Detection.

Olfaction begins with the detection of odorant molecules. Studies of olfactory behavior and physiology suggest that animals can have very low sensitivity thresholds for the detection of odors, such as in the insect pheromonal system (Hansson, 1995). Animals may need to detect hundreds or thousands of the many odor molecules present in the environment. The problem of detecting such a large range of possible chemosensory cues is solved by utilizing a large number of specialized ligand-binding proteins with variable specificities. In this aspect, olfaction is quite different from non-chemical senses such as vision and audition. Indeed, light or sound frequency is a continuous variable and can be defined along a linear scale. Frequency, intensity and perhaps phase can each be defined along a linear stimulus axis, and the resulting stimulus space is fairly low-dimensional. The set of behaviorally significant odor molecules, however, cannot be easily projected on any low-dimensional axis or surface. Odorants have many discrete characteristics which are often not continuous: presence or absence of a methyl group, three-dimensional steric structure, etc. If each characteristic must be represented by a separate axis in odor stimulus space, then the resulting space must be high-dimensional. Such a stimulus space creates difficulty for the experimenter (it is difficult to sample enough stimuli) and for the theorist (it is difficult to devise an appropriate computational strategy). The nervous system, however, appears to have found a reasonable solution for classification and recognition.

Odor discrimination and recognition.

Detection of molecules occurs with the olfactory receptor neurons which are positioned to be accessible to the airborne or aqueous odorants in the environment. Discrimination and recognition of known odorants most likely occurs in the central nervous system. The presence of odorants which are important to the animal must be extracted from the background, and "odor objects" identified. An odor may be monomolecular or a complex blend of odorant molecules in which both the identity and

(usually) the relative concentrations of the components are important for source recognition (e.g., kin recognition). In moths, for example, both the identity and the relative concentrations of the pheromone components are important for eliciting approach behavior in males of the appropriate species (Hansson, 1995; Hildebrand, 1996).

In this thesis, we study how odor identity is encoded using both monomolecular odorants and blends. It is currently not clear, however, whether monomolecular odorants and blends are treated any differently by the nervous system or are necessarily perceptually different. Both blends and monomolecular odorants are likely to activate a large number of peripheral receptors, since receptor neurons are broadly tuned (Gesteland et al., 1963; Getchell and Shephard, 1978a; Revial, 1982; Sicard and Holley, 1984; Firestein et al., 1993). Some behavioral evidence suggests that blends may be treated as a separate object from the components of which it consists. In the terrestrial slug, for example, an avoidance behavior may be learned for a blend of two odorants, but such behavior is not generalized for the components odorants themselves (Hopfield and Gelperin, 1989). On the other hand, humans are known to be capable (with some experience) of discriminating individual components of blends as complex as the odors of perfumes, wines, and food. Thus mechanisms must exist both for discriminating individual (known) components from a blend and also for "binding" blend components into a discrete object.

Concentration detection and concentration invariant representation.

The olfactory system must balance sensitivity to odors with the ability to identify an odor over a range of concentrations. In nature, the concentration of an odorant might range over several orders of magnitude, depending on factors such as wind speed, turbulence, and how long ago a scent had been left by another animal, for example. Olfactory nervous systems may handle this by gain control mechanisms, such as adaptation in the peripheral nervous system (e.g., Getchell and Shepherd, 1978b), or by forming concentration-invariant representations at the central level (Hopfield, 1991). In some cases, however,

concentration information may be crucial to tasks such as object localization. In other cases, the absolute concentration is not important but relative ratio of components must be determined, as is the case for the induction moth pheromonal behaviors (Hansson, 1995; Hildebrand, 1996). In moths, many species use the same components, but in different ratios. Thus concentration information must neither be thrown away nor allowed to interfere with odorant identification.

Olfactory coding.

The study of sensory coding is one of assigning meaning to the activity of neurons in terms of stimulus features. A "neural code" is a representation of information about the stimulus in the measurable neural activity. To be designated a neural code, the activity should be correlated with the stimulus, contain information about the stimulus, and this information should be available and computationally useful to the animal itself, not only available to an observer, such as the physiologist. A "code" should be decodable, ideally both by an observer and the neural structures which receive the information. With respect to an observer, it should allow reconstruction or identification of the stimulus features it encodes, given enough knowledge about the activity (e.g., mean firing rates, distribution over neuronal ensembles, spike timing, relationship of activity between different brain areas, etc.). With respect to the receiving, or downstream, neural structures, the code should allow the transfer of specific information about the stimulus, possibly transformed or processed in some way. Altering the neural code at one neural level should alter the stimulus representation at the next.

A number of coding schemes appear to be available to the olfactory systems across many animals. We will briefly outline these ideas, and direct the reader to the remainder of this chapter for details on the structure and physiology of specific olfactory systems, as well as some explicit models of olfactory computation (Sections III and IV).

Coarse, distributed coding.

In generalist olfaction, most neural responses at the periphery and in the central nervous system are broadly tuned. One must trade off specificity with the range of molecules to which sensitivity is required. It would require an impossibly large number of receptors if each one were specific for only one type of molecule or epitope. In addition, there are far too many monomolecular odorants and combinatorial blends to represent each one with the specific activity of a single central neuron, or even a very small, exclusive set of neurons. Instead, it seems clear from mapping and imaging experiments (Cinelli and Kauer, 1990; Cinelli et al., 1995b; Friedrich and Korsching, 1997; Joerges et al., 1997; Kauer, 1991) that many neurons participate in the representation of any given odor stimulus, and each neuron participates in many different representations.

Spatial mapping.

A remarkable feature of the olfactory system, especially in vertebrates, is the ordered spatial mapping of responses to odor stimuli. Specific peripheral olfactory receptor neurons project to specific glomeruli in the olfactory bulb, which implies at least some level of functional mapping (Ressler et al., 1993; Vassar et al., 1993; Mombaerts et al., 1996). Functional imaging and physiology reveal that activity occurs in the olfactory bulb and antennal lobe in a highly patterned, odor-specific way (Cinelli et al., 1995b; Friedrich and Korsching, 1997; Joerges et al., 1997; Kauer, 1991). These findings indicate that information about odor identity is contained in the identity of the neurons activated by a stimulus. However, it seems unlikely that the spatial positioning of such activity is "decoded" by the animal itself, since neuronal location, per se, does not appear to be a parameter that is transmitted to the next level. Relative positioning of odor-evoked activity in the nervous system may be important for computational tasks, such as lateral inhibition between similarly tuned neurons, or may simply be due to developmental causes. Pending

a better understanding about whether, or how, these ordered spatial patterns may be relevant to downstream processing, they should not necessarily be considered a "neural code" for olfactory information.

Coding with time.

The temporal patterning of olfactory responses is readily apparent in many olfactory systems, and is particularly striking in the locust olfactory system. Temporal activity patterning takes many forms, some of which occur simultaneously, and are non-exclusive. Slow temporal patterning of action potential firing occurs in not only insects (Burrows et al., 1982; Kanzaki et al., 1989; Laurent and Davidowitz, 1994; Laurent et al., 1996), but also in mammals, frog, fish, and amphibian (Chaput and Holley, 1980; Kauer, 1974; Kauer and Shepherd, 1977; Meredith, 1981; Meredith, 1986; Meredith and Moulton, 1978). These slow patterns are a progression composed of both excitation and suppression of variable duration and interval (on the time scale of several hundred milliseconds to seconds) and may provide a more sophisticated version of the coarse distributed encoding described above, wherein the activity is dynamically evolving over time rather than based on static mean firing rates.

Of central importance to this thesis work, oscillatory neural synchronization is a widespread phenomenon in the olfactory systems across several phyla, from mollusc to mammal. Oscillatory activity itself can take on several forms, which may have separate physiological functions. First, a 4-12 Hz rhythm, designated as theta rhythm, is found in the olfactory bulb and olfactory cortex of vertebrates, and appears to be related to the sniffing frequency, at least in small mammals (Buonviso et al., 1992; Chaput and Holley, 1980; Chaput, 1986; Sobel and Tank, 1993). They are often coherent with the rhythms of similar frequency in entorhinal and hippocampal formation areas (Freeman, 1975). Work in the hippocampus suggests that the theta rhythm may be involved in memory processes or spatial coding (Burgess et al., 1994; O'Keefe and Recce, 1993). Theta rhythm in

olfactory areas is not directly known be related to the formation of olfactory memories or recall. There is no evidence yet in insect systems suggesting a theta-like rhythm, although in a mollusc olfactory system, very slow (~1Hz) oscillations are known to occur (Gelperin and Tank, 1990; Delaney et al., 1994; Kleinfeld et al., 1994).

Second, concurrent with theta oscillations are the "fast" oscillations, also called gamma (γ) oscillations. These fast oscillations range from 40 to 70 Hz in mammalian systems and from 20 to 30 Hz in insect systems. This fast oscillatory activity may be involved in olfactory sensory encoding (this thesis; Laurent and Naraghi, 1994; Laurent and Davidowitz, 1994; Laurent et al., 1996; Wehr and Laurent, 1996; Wehr, 1999) and in contextual olfactory learning (Viana Di Prisco and Freeman, 1987) (see Section IV). In other sensory systems, neural synchronization is thought to be involved in such varied perceptual and cognitive tasks as binding, segmentation, and attentive behavior (see Section V).

In summary, a number of coding strategies are utilized by the olfactory systems of insects, amphibians, reptiles, molluscs, and mammals. In the locust olfactory system, we have proposed the hypothesis that odorant identity is encoded by a distributed and dynamic code of oscillatory sequences of synchronized neural ensembles (Laurent and Davidowitz, 1994; Laurent et al., 1996; Laurent, 1996). These oscillatory sequences of activity across a neural population are correlated with odor stimulation and are odor-specific (Wehr and Laurent, 1996). It is one purpose of this thesis to test whether one temporal feature of this coding hypothesis, specifically neural synchronization, is necessary for downstream decoding, and thus truly a component of the neural code.

III. The Olfactory System.

The Periphery.

Odors are detected by olfactory receptor neurons (ORNs) located in the nasal epithelium in vertebrates, and in olfactory sensilla on the antennae of insects. The ORNs are accessible from the environment by direct inspiration of odor-laden air through the nasal cavity in vertebrates, or through pores in the cuticle of insect sensilla. These sensory neurons are highly specialized for chemoreception with ligand-binding receptor proteins in their cell membranes. These receptor proteins signal the detection of odorants by initiating signal-amplifying second-messenger cascades via G-protein activation. Finally, second-messenger sensitive ion channels depolarize the membrane, causing it to fire action potentials. In addition, several types of odorant binding proteins (OBPs) are secreted by the ORNs into the epithelial mucus or sensillar lymph which aid in the transport of the (typically hydrophobic) airborne odorants to the membrane bound olfactory receptor proteins.

Odorant Binding Proteins.

In insects, a large family of odorant biding proteins has been found (Vogt and Riddiford, 1981; Gyorgi et al., 1988; Raming et al., 1989; Pelosi and Maida, 1990; Krieger at al. 1991, 1993; McKenna et al., 1994; Pikielney et al., 1994). Pheromonal binding proteins (PBPs; Vogt and Riddiford, 1981) and general odorant binding proteins (GOBPs; Breer et al., 1990b; Vogt et al., 1991a,b) have been identified in moth. PBPs are abundant 15 kilodalton soluble proteins found in the sensillum lymph of male antennae, initially identified in the silkmoth, *Anteraea polyphemus*. They have highly specific ligand binding affinities for the components of the female sex pheromone. PBPs and GOBPs together form a family of proteins with helical tertiary structure (Du and Prestwich, 1995).

A family of vertebrate OBPs has been identified in the nasal mucosa (Pelosi et al., 1982; Pevsner et al., 1985; Pevsner and Snyder, 1990). These ~20 kilodalton proteins

appear to be unrelated to insect OBPs (Pevsner et al., 1988; Pelosi and Maida, 1990). Their ligand specificities also appear to be broader than those reported for insect PBPs or GOBPs.

Odorant binding proteins are likely to function primarily as transporters for odorant molecules through the lymph or mucus to the ORNs. They may also have a role in rapidly deactivating the odorants through binding, and then aiding in enzymatic degradation. Pheromone-specific esterases have been found in insect lymph (Vogt and Riddiford, 1981). Binding and rapid degradation processes may be particularly important in systems where very high sensitivity (resulting in saturation at moderate concentration) of the ORNs to odorants (such as sex pheromones) may be required for appropriate behavior.

Olfactory Receptor Proteins.

A key element in odor detection and transduction is the ligand-binding olfactory receptor protein. The binding affinities, sensitivities, selectivities and expression patterns of the receptor proteins must determine the ORN physiological properties. A family of putative olfactory receptor genes has been identified and cloned in the vertebrate nervous system, first in rat (Buck and Axel, 1991), and subsequently in mouse (Ressler et al., 1993) and fish (Ngai et al., 1993b). However, an equivalent family has yet to be reported for insects or other arthropods. Olfactory receptor proteins have also been reported in another invertebrate, the nematode *Caenorhabditis elegans* (Troemel et al., 1995), and their role in chemosensation directly demonstrated (Zhang et al., 1997).

Identification of the vertebrate olfactory receptor genes was accomplished through a cloning strategy based on the observation that ORN odor responses depend on second messenger cascades, suggesting that the receptors are probably seven-transmembrane domain G-protein coupled receptors (Pace et al., 1985; Lancet, 1986). The isolated genes define a family of proteins whose members number approximately 1000 in rat (Buck and Axel, 1991), and 30-100 in fish (Ngai et al. 1993). They are expressed almost exclusively

in the ORNs in olfactory tissue, suggesting that they are involved in olfaction. The large number of putative receptor genes correlates with the expectation that a large range of ligand binding specificities would be required for the task of general olfaction. Experiments utilizing *in situ* hybridization of probes for the mRNA of the putative receptors suggested that individual ORNs express only one or (at most) a few receptor protein types (Chess et al., 1994). Studies of the distribution of receptor gene expression over the whole olfactory epithelium showed that a broad organizational structure exists, consisting of at least four distinct spatial zones in which different sets of odorant receptor genes are expressed (Ressler et al., 1993; Vassar et al., 1993; Strotman et al., 1992). Within each zone, ORNs that express a particular gene are randomly distributed, and thus each zone contains a mosaic of ORNs expressing different genes.

In addition to the gene family above, two other families of putative pheromonal receptor proteins (unrelated to each other) expressed in the vertebrate vomeronasal organ have been reported (Dulac and Axel, 1995; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). These two gene families appear to be unrelated to the 'general' olfactory receptors, or other G-protein coupled receptors.

The molecular biological and anatomical evidence supports the hypothesis that the cloned genes are the olfactory receptors, and functional expression assays furthermore suggest that the putative general olfactory receptors indeed transduce odors. Zhao et al. (1998) overexpressed a putative receptor gene in rat olfactory epithelium *in vivo* by infecting the tissue with a recombinant adenovirus vector containing one olfactory receptor gene. This method ensured that the rest of the olfactory transduction machinery was intact and functional. The summed extracellular potential, or electro-olfactogram (EOG), of the epithelia in response to stimulation with a battery of odorants was measured. Epithelia infected with this receptor gene showed enhanced EOG responses to a small subset of odors (e.g., octyl aldehyde, and somewhat to chemically very similar aldehydes, hept-, nonyl- and decyl-aldehyde) compared to control animals. These results strongly suggest

that the putative receptor genes encode proteins which can bind odors and transduce ligand binding into detectable electrical response in ORNs.

Second messenger systems and ion channels.

Odorant transduction appears to utilize G-protein coupled second-messenger cascades to amplify and transmit ligand detection (Schild and Restrepo, 1998). In vertebrates ORNs, odor stimulation and ligand binding by receptors activates an olfactory specific G-protein (G_{olf}) (Jones and Reed, 1989; Menco et al., 1992), which then turns on adenylyl cyclase, leading to a production of cyclic adenosine monophosphate (cAMP) (Pace et al., 1985). The rise in cAMP then gates directly a non-specific cation channel (Nakamura and Gold, 1987; Dhallan et al., 1990) and indirectly a Ca^{+2}-activated Cl^- current (Kleene and Gesteland, 1991. After odor stimulation, the cation channel allows an influx of a mixture of Na^+, K^+ and Ca^{+2}; calcium influx then activates the Cl^- current. The chloride gradient for ORNs leads to a chloride reversal potential close to zero millivolts (i.e., much less negative than the resting potential) (Trotier, 1986; Firestein and Werblin, 1989), and thus the Cl^- current contributes to the depolarization of the membrane. The resulting depolarization triggers action potentials. The cation channel, a cyclic nucleotide-gated (CNG) channel (Nakamura and Gold, 1987; Dhallan et al., 1990), is 70% homologous to the CNG channels found in photoreceptors (Kaupp, 1991).

In insects, however, the pathway generally appears to involve inositol triphosphate (IP_3) and diacylglycerol production instead of cAMP. Stimulation of antennal homogenates of moths or cockroaches with the appropriate sex pheromone leads to a fast (~50ms) and transient increases of IP_3, but not of cAMP (Breer et al., 1990a; Boekhoff et al., 1993). The time course of IP_3 production is similar to the time course of the odor-evoked electrical conductances in intact ORNs.

Some observations suggest that both pathways may in fact exist in vertebrates (Sklar et al., 1986; Boekhoff et al., 1990b; Ronnett et al., 1993) and in crustaceans (Fadool

and Ache, 1992; Michel and Ache, 1992). While it has been suggested that selective activation of these pathways by different odors could subserve complex computational interactions within a single ORN (Shepherd, 1991), such effects have not yet been convincingly demonstrated.

Second messenger cascades are primary sites for effecting sensory adaptation, the reduction of the neural response to a maintained or repeated stimulus, of the ORNs. In insects, prolonged odor stimulation leads to an increase in cGMP levels, which may induce a downregulation of the odor-sensitive cation conductances, for example (Zufall et al., 1991; Stengl et al., 1992a; Ziegelberger et al., 1990). In vertebrates, candidate mechanisms for adaptation involve kinase phosphorylation of adenylyl cyclase or other second messenger proteins, calcium-calmodulin or carbon monoxide regulation of the CNG channels (Kramer and Siegelbaum, 1993; Breer, 1994; Leinders-Zufall et al., 1995).

Thus the chain biochemical reactions form the basis of the odor responses of the ORNs. Their characteristics endow the ORNs with their particular specificities and sensitivities, which are, unfortunately, still ill-characterized physiologically. Olfactory receptor proteins are responsible for the fundamental step of detecting specific molecules, and further understanding the relationship between their molecular structure and function is required for a complete understanding of odor encoding. The second messenger cascades and CNG channels provide ample opportunity for regulation of the odor responses, such as the amplification of very weak stimuli or adaptation to strong stimuli, in preparation for the next step in odor processing: the olfactory bulb (vertebrates) or antennal lobe (insects).

Olfactory bulb and antennal lobe.

Olfactory receptor neurons send axons from the periphery to the first relay of the olfactory system, which in vertebrates is called the olfactory bulb (OB) and in insects is called the antennal lobe (AL). These analogous structures are very similar. First, in every

vertebrate and insect species studied, both the OB and AL input layers are subdivided into small regions of neuropil called glomeruli. Glomeruli appear to be important for the organization of the inputs from the periphery. It has often been suggested that glomeruli are functional units (Leveteau and MacLeod, 1966), but this has yet to be clearly demonstrated. Second, the types of neurons and their connectivity are similar in the OB and AL. In both vertebrates and insects, a key feature is the reciprocal circuitry between excitatory and inhibitory interneurons. Third, the physiological function and odor responses have similar features (see Section IV), including the oscillatory neural synchronization which is the focus of this thesis.

Structure of the vertebrate olfactory bulb.

The vertebrate olfactory bulb is a conspicuously layered structure, leading some to consider it a model system for the cortex (Shepherd, 1970). Inputs arrive at the outermost glomerular layer. Below this is the external plexiform layer which is a principal site for local synaptic interactions. The next two layers are named for the neurons whose cell bodies occupy them, the mitral cell layer and granule cell layer.

Mapping from the epithelium to the bulb.

The axons of the ORNs project to the olfactory bulb with stunning precision. *In situ* hybridization of probes for olfactory receptor mRNA, in addition to labelling ORN somata in the epithelium (Buck and Axel, 1991; Chess et al., 1994), also labelled their axon terminals in the olfactory bulb (Vassar et al., 1994; Ressler et al., 1994). Probes for a particular subset of genes only labelled a few glomeruli in a stereotyped pattern across individuals, suggesting that the ORNs expressing those genes converge to small, highly specific subsets of glomeruli. In a beautiful set of experiments, ORNs expressing a particular putative receptor gene were specifically labelled with a tau-lac-z fusion protein,

which allowed visualization of the entire projection path of ORN axons from epithelium to bulb (Mombaerts et al., 1996). The labelled ORNs were scattered in the epithelium, but their axons formed a tight fascicle which projected precisely and directly to its target site in the bulb, a pair of glomeruli (one medial, one lateral).

These molecular biological results refine earlier evidence from tracing studies, which had suggested a mapping from epithelium to bulb involving a significant degree of both convergence and divergence of ORNs onto the glomerular layer of the OB (Kauer, 1980, 1981; Land 1973; Land and Shepherd, 1974; Costanzo and O'Connell, 1978; Astic and Saucier, 1986). Injections of horseradish peroxidase (HRP) dye into the bulb (retrograde labelling) or into the epithelium (anterograde labelling), or degeneration experiments, indicated a mapping that corresponded to anterior-posterior and medial-lateral axes. The more restricted the injections into, or ablations of, the peripheral tissue, the more restricted the resulting maps in the bulb. Subsections of epithelium appeared to diverge to multiple glomeruli in the bulb, and different areas of epithelium appeared to sometimes converge onto overlapping sets of glomeruli. These results, however, can now be explained by the molecular biological evidence that, at the periphery, ORNs expressing different genes are intermixed, and ORNs expressing the same gene are spread out over a rather large zone in the epithelium.

Neurons of the olfactory bulb.

The principal excitatory neurons of the OB are the mitral cells, first described by Ramon y Cajal (1911). The primary neurotransmitter of the mitral cells is thought to be glutamate, which is abundant in the OB (Popov et al., 1967; Jahr and Nicoll, 1982a; Nicoll, 1971). Each mitral cells has an axon that projects out of the OB to higher olfactory centers via the lateral olfactory tract (LOT), and two distinct sets of non-spiny dendrites. The primary dendrites invade the outermost, glomerular layer of the OB and arborize the extent of the glomerulus with a tuft of branches. In the vertebrate species most studied (rodents

and rabbits), each mitral cell appears to have a single primary dendrite which arborizes in only one glomerulus. However, in several 'lower' vertebrates, output neurons may be multiglomerular, projecting to several (2-10) glomeruli (goldfish: Satou, 1989; turtle, Mori et al., 1981a; frog: Jiang and Holley, 1992). The dendritic tufts extend throughout the glomeruli, and receive direct input from the ORNs. The basal dendrites have arborizations which are limited to the external plexiform layer. A second group of output neurons, called tufted cells, also receive direct input, but are distinct from typical mitral cells in the extent of their dendritic arborizations (Shepherd, 1990) and may thus process somewhat different information than the mitral cells. For simplicity, and consistent with the convention of most authors, we will refer to the entire output neuron population as mitral cells, since most observations apply to both cell types.

The basal dendrites of the mitral cells contact a population of local interneurons in the external plexiform layer, the granule cells. The granule cells are small, spiny, axonless neurons whose dendrites are also limited to the external plexiform layer. They interact with the basal dendrites of the mitral cells via dendrodendritic synapses, a type of synapse that was identified first here in the olfactory bulb. These connections are characterized by reciprocal synaptic profiles (that is, excitatory, mitral-to-granule synapses and inhibitory, granule-to-mitral synapses are found in close conjunction) identified anatomically in electron microscopic studies (Rall et al., 1966; Reese and Brightman, 1965; Reese, 1966; Price and Powell, 1970). Pharmacological and physiological evidence suggests that the inhibitory neurotransmitter used by the granule cells is γ-aminobutyric acid (GABA) (Halasz and Shepherd, 1983; Isaacson and Strowbridge, 1998; Jahr and Nicoll, 1982a; Nicoll, 1971; Ribak et al., 1977; Wellis and Kauer, 1993, 1994). Granule cells are also the principal target of feedback fibers from piriform cortex (Haberly and Price, 1978).

Mitral cells also receive input from the local interneurons in the glomerular layer, the periglomerular cells (PG). Periglomerular neurons are small, short-axon neurons which may subserve interactions either within a glomerulus and/or across two or more

neighboring glomeruli (Pinching and Powell, 1971a; Schneider and Macrides, 1978). PGs are thought to use GABA as a neurotransmitter (Ribak et al., 1977), although their effect may be excitatory, due to modified chloride gradients across mitral cell dendrites (Siklós et al., 1995).

Thus the olfactory bulb uses the neurotransmitters typical of the vertebrate brain, glutamate (and possibly aspartate) and GABA. A variety of neuromodulators, including peptides and biogenic amines, are also found in the OB (Halasz and Shepherd, 1983) and may also contribute to OB function. Centrifugal fibers, for example, are associated with norepinephrine, serotonin, and acetylcholine (Shepherd, 1990).

Structure of the insect antennal lobe.

The insect antennal lobe is structurally analogous to the olfactory bulb, for it receives chemosensory neuron input from the main peripheral olfactory organ, the antenna. All insect species with an olfactory sense (odonats, or dragonflies, for example, have no antennae and no antennal lobes) have antennal lobes with a glomerular neuropilar structure, and two main classes of neurons: an excitatory class (the output, or projection, neurons) and an inhibitory class (the local interneurons). The antennal lobe of the locust is a radial structure, with the somata forming a peripheral-most layer, a glomerular neuropil where ORNs project, and an interior coarse neuropil formed by the primary processes and branches of the antennal lobe neurons.

Afferent input and glomerular structure.

In the locust, the glomeruli appear to number ~1000, and are each ~10-30 micrometers in diameter (Ernst, Boeckh and Boeckh, 1977; Laurent and Naraghi, 1994). The number of glomeruli in the antennal lobe of other insects range from 50 to 200 in honeybee, fly and moth species (Masson, 1990). The glomerular neuropil in locust is sometimes referred to as "microglomerular," since the glomeruli are smaller than those in

most other insects (which range up to 20-200 micrometers). In hemimetabolous (non-metamorphosing) insects such as the cockroach and locust, the glomeruli are distributed throughout the lobe, while in holometabolous insects (e.g., bees, flies, moths), the glomeruli are arranged in a single layer around the coarse neuropil, reminiscent of the vertebrate bulb. Glomeruli in some insect species (e.g., honeybee, fly, roach) are identifiable, and invariant between individuals (Masson, 1974; Arnold et al., 1984; Chambille and Rospars, 1985; Chambille et al., 1980; Rospars, 1983; Rospars and Chambille, 1981). In honeybees there are differences which depend on caste (i.e., worker, drone, or queen)(Arnold et al., 1984, 1985, 1988), suggesting a relationship between the glomerular structure and behavioral and/or hormonal function.

In some species, there is a sexually dimorphic glomerulus, known as the macroglomerular complex (MGC), which is specifically involved in sexual pheromone detection and processing. This sexual dimorphism has received a great deal of attention in moth and cockroach, where some of the sex pheromone components have been identified. The macroglomerular complex is a large, male-specific, distinct area of neuropil near the entrance of the antennal nerve into the AL. The MGC is the exclusive target of pheromone responsive receptor neurons (Hildebrand et al., 1980; Schneiderman et al., 1982; Distler et al., 1986; Koontz and Schneider, 1987), and the AL neurons arborizing within it respond to pheromonal stimulation (Matsumoto and Hildebrand, 1981; Burrows et al., 1982; Boeckh and Selsam, 1984; Christensen and Hildebrand, 1987b; Kanzaki et al., 1989). Furthermore, the MGC can be divided into anatomical and functional subcomponents (Boeckh and Tolbert, 1993; Ernst and Boeckh, 1987; Hansson et al., 1991; Hansson et al., 1992). The MGC, then, is one case where a function can be assigned to a particular glomerulus. An MGC does not appear to be present in the locust; in fact, there do not seem to be any sexual dimorphisms in the olfactory locust brain.

In the locust, approximately 50 to 90 thousand antennal afferents project to the ipsilateral AL (Masson and Mustaparta, 1990). There are no known contralateral

projections in locust, though a few have been found in other species (Masson and Mustaparta, 1990). Each receptor axon can branch and terminate in 1-3 different glomeruli (Ernst, Boeckh and Boeckh, 1977). Most of the afferent input to the antennal lobe is olfactory (Masson and Mustaparta, 1990; Kaissling, 1971), although a minority of fibers are sensitive to mechanical stimuli, or to heat or humidity (Altner et al., 1977). A majority of the antennal nerve axons, likewise, directly innervate the antennal lobe. However, a minority of the antennal nerve axons project instead to the posterior part of the deutocerebrum, the dorsal lobe, which receives mixed sensory and motor innervation mainly from the most proximal antennal segments (Masson and Mustaparta, 1990).

Projection neurons.

Projection neurons, identifiable by their axonal projection to the protocerebrum, number approximately 830 in the locust (Leitch and Laurent, 1996). Each PN has a soma located near the surface of the antennal lobe. The PNs in locust are multiglomerular: within the antennal lobe, a primary process branches into 10-20 neurites which radiate out to as many different peripheral glomeruli and eventually terminates as dense tufts of fine branches (Laurent and Naraghi, 1994).

In locusts, the axons of the PNs gather into a single tight bundle (Leitch and Laurent, 1996) which forms the antennoglomerular tract (AGT). They project first to the ventral area of the mushroom body calyx, where each PN axon gives rise to a few widely dispersed, sparse, varicose collateral branches onto the dendrites of the mushroom body intrinsic neurons (Kenyon cells, see below). The main axon of each PN continues on to terminate in the lateral lobe of the protocerebrum. In some species (honeybee, cockroach), one group of two output tracts from the antennal lobe terminates in both the MB and lateral lobe, while a second group of tracts from the antennal lobe terminates only in the lateral lobe (Mobbs, 1982; Masson and Mustaparta, 1990).

Local interneurons.

In the locust, the axonless local interneurons (LNs) of the AL are distinct from the projection neurons in their morphology and physiology. The local interneurons number approximately 300, and thus account for roughly one-quarter of the AL neurons. LN dendritic fields are large (several hundred microns across) and complex. Each LN has many branches which densely arborize within the entire antennal lobe neuropil, presumably making contacts in a large number of glomeruli. In other insect species, the morphology of local interneurons tends to be multiglomerular, but with varying levels of restricted arborization. They vary from arborizing within most glomeruli (moth: Homberg et al., 1988; Matsumoto and Hildebrand, 1981) to arborizing only within a subset of glomeruli (cockroach: Ernst and Boeckh, 1983; honeybee: Flanagan and Mercer, 1989).

The local interneurons in locust are GABAergic, as in other insects (Masson and Mustaparta, 1990; Hoskins et al., 1986; Waldrop et al., 1987; Leitch and Laurent, 1996) and have a direct inhibitory effect on the PNs, as will be shown here (MacLeod and Laurent, 1996).

The local interneurons are likely to serve several functions which in vertebrate olfactory bulb are divided among two or more inhibitory neuron populations. These functions may include inter- and intraglomerular interactions (presumably like the periglomerular neurons in vertebrates) and lateral and self-inhibition of projection neurons (like the granule cells in vertebrates).

Antennal lobe connectivity.

Synaptic connectivity in the antennal lobe is complex. Since all contacts appear to be made in the glomerular neuropil, there is no clear anatomical separation of input synapses and those involved in intrinsic processing, as found in the vertebrate system. There are biochemical (Sanes and Hildebrand, 1976c; Sanes et al., 1977; Hildebrand et al., 1979) and physiological data (Waldrop and Hildebrand, 1989) to suggest that the

neurotransmitter of the PNs is probably acetylcholine. Electron microscope immuno-histochemical study of the locust antennal lobe indicates that GABAergic processes (presumably the LNs) make output synapses on to both GABAergic and non-GABAergic processes, and receive input from both GABAergic and non-GABAergic processes (Leitch and Laurent, 1996). In addition, non-GABAergic processes make contacts with one another. These data suggest that all combinations of connectivity are possible, including contacts between LNs, between PNs, and reciprocal feedback loops between LNs and PNs. In addition, serial connectivity was found; for example, synaptic contact from a non-GABAergic profile onto a second non-GABAergic profile, which in turn contacts yet another GABAergic profile. The data are also consistent with both PNs and LNs receiving direct input from the ORNs (monosynaptic) as well as feedforward, indirect input (polysynaptic).

This broad range of connectivity via multiple ultrastructural pathways suggest that the antennal lobe circuitry can support complex, dynamic activity. The PNs are thus, most likely, not simple relay links between the antenna and mushroom body, but instead are participants in the generation of complex activity patterns characteristic of PN odor responses (Laurent and Davidowitz, 1994; Wehr and Laurent, 1996).

Other identified neurons with ramifications in the antennal lobe.

Few neurons which may provide centrifugal input to the antennal lobe have been found. Pairs of serotonergic neurons have been identified, one in each lobe, in moth (Kent et al., 1986; Sun et al., 1993), in cockroach (Salecker and Distler, 1990), and in honeybee (Rehder et al., 1987). Immunohistological staining revealed anti-serotonin positive profiles presynaptic to other AL neurons, suggesting that serotonin may have a functional influence (Salecker and Distler, 1990). In addition, a group of LNs has been shown to be dopaminergic in roach (Distler, 1990b). Two types of octopaminergic neurons in antennal lobes have been identified. One type in the locust has branches mainly in the antennal

lobe, while a different type in honeybee has branches in the antennal lobe and extensively in the protocerebrum (Braunig, 1991; Hammer, 1993; Kriesel et al., 1994).

Staining for NADPH diaphorase, generally indicative of nitric oxide production, had been found in cell bodies in the AL, whose primary processes project toward the AL glomerular neuropil, suggesting that NO may play a role in local olfactory processing (Müller and Bicker, 1994). These results agree with previous reports of NO synthase in locust (Elphick et al., 1993) and other insect antennal lobes (Müller and Buchner, 1993; Müller, 1994).

Several differences in the anatomy and structure of the AL of locust versus other well studied species exist. In honeybees and roaches, the output of the AL forms two or more tracts, which mostly project to the mushroom body calyx and somewhat to lateral lobe (Mobbs, 1982). Also, PNs which innervate the MGC project to a different site in the protocerebrum from those PNs which innervate ordinary glomeruli (Homberg et al., 1988; Kanzaki et al., 1989; Kraus, 1991). The PNs in other species can be either uniglomerular or multiglomerular, but the uniglomerular type are predominant (Masson and Mustaparta, 1990), both in ordinary glomeruli (Ernst and Boeckh, 1983; Kong and Strausfeld, 1989) and in MGC (Ernst and Boeckh, 1983; Homberg et al., 1988). In contrast, no uniglomerular PNs have been found in locust. However, the physiological responses of uniglomerular PNs in species where they are found are not qualitatively different from multiglomerular PNs (Kanzaki et al., 1989). The glomeruli are larger and more readily identifiable in other species than in locust.

Higher olfactory centers.

Another feature common to both vertebrate and insect olfactory systems is that the primary relay further projects to a secondary olfactory area which is closely linked with

learning and memory: the piriform cortex in vertebrates, and the mushroom body in insects.

Principal site in vertebrates is the piriform (or prepiriform) cortex

The output neurons of the olfactory bulb form an axonal bundle called the lateral olfactory tract (LOT), which projects directly to several cortical areas without relaying through the thalamus, like the fibers carrying sensory input of most other modalities. The largest region of primary olfactory cortex is called the piriform cortex. The piriform cortex is paleocortical (phylogenetically old cortex) with a three-layered structure rather than the six typical of the neocortex (Haberly, 1985).

Neurons of the piriform cortex.

The principal neurons of the piriform cortex, like those of the neocortex, are the pyramidal cells. These neurons receive direct afferent input from the olfactory bulb. Morphologically these cells are similar to pyramidal neurons in other areas, with spiny apical and basal dendritic trees. Two pyramidal cell types, superficial and deep, can be morphologically defined based on the position of their cell bodies and the length of the apical dendrites. There is an additional excitatory cell type called semilunar, or pyramid-like, neurons, which have no basal dendrites (Haberly, 1985).

There are several types of GABAergic, inhibitory interneurons in the piriform cortex. These include multipolar cells, small globular cells, and superficial horizontal cells. The more superficially positioned of these neurons, the horizontal cells and small globular cells, have been hypothesized to have a feedforward inhibitory function (Haberly, 1985). This idea is supported by indirect physiological evidence (Haberly, 1973; Satou et al., 1983; Tseng and Haberly, 1988). The multipolar cells, on the other hand, which are found

in all but the most superficial layers, are in a position to play a role in feedback inhibition (Biedenbach and Stevens, 1969; Haberly, 1985).

Afferent inputs to the piriform cortex.

The LOT, containing the axons of the mitral cells, spreads obliquely across the surface of the piriform cortex in the superficial part of layer 1, sending off collatorals horizontally within the layer. Synaptic evidence suggests that each axon makes numerous connections along its length onto the pyramidal neurons. The extent of the divergence of the afferent input appears to be quite significant, since the estimated ratio of OB mitral cells to piriform pyramidal neurons is estimated to be ~1:15 (in lower mammals, Haberly, 1990) and each afferent contacts multiple postsynaptic cells.

Retrograde labelling studies show that the piriform cortex receives input from broadly distributed areas in the bulb, although some patterning can be defined (Haberly and Price, 1977; Skeen and Hall, 1977; Scott et al., 1980). While more advanced techniques might reveal a more refined topography, as occurred with the molecular biological dissection of the bulb, it appears that there is, at least, no point-to-point topography between olfactory bulb and cortex. Thus the precise topography of the bulb, in which the terminals of the ORNs are segregated by receptor protein gene types, appears, at this point, to be blurred, if not lost entirely, in the olfactory cortex.

Association connections and outputs of the piriform cortex.

Pyramidal neuron axons project mainly via tangentially distributed association fibers to other areas of cortex, both within the piriform cortex and with neighboring olfactory areas. Axon collatorals radiate in all directions from the pyramidal cell body, and make contacts with other neighboring pyramidal neurons in layer 1 (strictly segregated from the more superficial LOT afferents in layer 1) and layer 2. Like the LOT afferents, each pyramidal neuron appears to contact a large number of post-synaptic targets,

contributing to the distributed nature of piriform cortical connectivity. Topographically, there is some tendency of the association fiber system to project from caudal to rostral (Haberly, 1985).

The piriform cortex sends projections to most other olfactory areas which receive direct input from olfactory bulb, including olfactory tubercle, olfactory peduncle, entorhinal cortex, insular cortex and amygdaloid cortex (Haberly and Price, 1978a; Luskin and Price, 1983b). Many of these areas also reciprocally project to the piriform cortex (peduncle, entorhinal, amygdaloid cortex). The output from piriform cortex occurs largely via associational projections to neighboring olfactory areas. The piriform cortex also makes a feedback projection to the olfactory bulb (Luskin and Price, 1983a,b), which may be an important component of olfactory processing. Physiological and dye tracing experiments established that the olfactory areas of the orbital and insular cortex receive input from the primary olfactory centers directly and via a thalamic relay (Clugnet and Price, 1987; Luskin and Price, 1983b).

Principal site in insects is the mushroom body.

A wealth of anatomical, genetic and behavioral data suggests that the mushroom body is an important area for olfactory learning and memory and for multimodal sensory convergence, two functions which are not mutually exclusive.

Structure of the mushroom body.

The mushroom body is so named for the mushroom-like shape of its neuropil. It consists of a cup-like dorsal region (the calyx), a stalk-like region (the pedunculus), and two (or more) distal putative output lobes, labelled α and β (and γ)(Kenyon, 1896; Pearson, 1971; Schürman, 1974; Mobbs, 1982; Boeckh and Ernst, 1987). The calyx can be divided into nested subregions, the lip, collar, and basal neuropil, which have been shown to receive distinct sensory input: the olfactory inputs branch primarily in the lip

neuropil, while visual inputs coming from the optic tract project primarily in the collar and basal neuropil (Mobbs, 1982). The projection neurons of the antennal lobe send their axons via the antennoglomerular tract to the calyx of the mushroom body. These axons have sparse, widely distributed varicose collaterals in the calyx which make en-passant synapses with the dendrites of the intrinsic neurons, the Kenyon cells (KCs) (Kenyon, 1896; Laurent and Naraghi, 1994; Leitch and Laurent, 1996; Masson and Mustaparta, 1990). Each PN probably contacts several hundred KCs.

The neuropil is defined by the processes of the intrinsic neurons, the Kenyon cells (Kenyon, 1896). The ~50,000 KCs have small somata located dorsally, each sending a single primary dendrite into the calyx. In the calyx, each KC has several spiny branches, which comprise a restricted dendritic field oriented radially. The KC axons form the pedunculus, which appears tightly bundled and nearly hexagonal in crosssection (Leitch and Laurent, 1996). Each axon bifurcates and sends one branch (or sometimes two) into each of the α- and β-lobes. Within these lobes the KC axons have short side branches, but otherwise do not arborize densely.

That the mushroom body neuropil may be further subdivided, and that Kenyon cells may not be isomorphic, has been revealed by studies of the patterns resulting from anatomical stains using traditional methods (Li and Strausfeld, 1997) and transcriptional reporter expression systems (Kaiser, 1993; Yang et al., 1995).

Extrinsic neurons

While the calyx has been considered the 'input' neuropil of the mushroom bodies, the α- and β-lobes have been labelled the 'output' neuropil. Kenyon cells contact several types of complex output and feedback neurons, which together we will refer to as the general class of 'extrinsic' neurons (Homberg, 1984; Li and Strausfeld, 1997; Schürmann, 1974; Gronenberg, 1987; Mobbs, 1982; Erber et al., 1987). Output neurons project to areas outside of the MB neuropil, and must be responsible for the transmission of

information beyond the MB. Feedback neurons make both input and output connections within the MB and probably play a role in intrinsic information processing.

Unlike the dense grouping of the somata of the Kenyon cells, the extrinsic neurons have somata that are scattered individually or in small groups intermingled with other somata throughout the protocerebrum (Mobbs, 1982; Erber et al., 1987). (Thus, of interest to the experimenter, no clearly defined cluster of extrinsic neuron cell bodies which would allow somatic impalement with certainty as to its identity, has yet been identified.)

While the olfactory input to the mushroom body is clearly via the AGT carrying the projection neuron axons, the output pathways from the MB are not as well defined. In honeybees, there are up to eight different tracts leading from the MB neuropil (Mobbs, 1982). These fiber bundles generally access the α- and β-lobes at two restricted points: one at the lateral margin of the α-lobe, and the other at the junction of the two lobes. The neurons travelling in these output tracts project to several well defined neuropils in the protocerebrum, including the lateral lobe and the central body. Other targets are more diffuse neuropils within the protocerebrum, such as those surrounding the mushroom body itself. In addition, a number of individual fibers independently innervate the lobes without entering any of these tracts (Mobbs, 1982).

Synaptic structure.

Olfactory information through the mushroom body, then, appears to flow from PNs to KCs to extrinsic neurons. However, two features of the synaptic organization may greatly complicate this connection scheme. First, the KCs appear to make excitatory connections with one another in the pedunculus between their axons, sometimes serially (Schürmann, 1974; Leitch and Laurent, 1996). Second, the complexity of the extrinsic neurons, both output and feedback, also suggests a much more elaborate functional circuitry.

Inhibitory interactions may be one important component of mushroom body olfactory processing. Immunohistochemical evidence for GABA in the locust mushroom body revealed a large number of immunopositive profiles throughout the calyx, pedunculus and both α- and β-lobes (Leitch and Laurent, 1996). The Kenyon cells themselves, however, do not appear to be GABAergic (Leitch and Laurent, 1996). Since the PNs are not GABAergic, it is most likely that at least some of the extrinsic neurons are indeed GABAergic. Thus, some of these neurons may function as inhibitory feedback circuits. In the calyx, synaptic contacts from presumed PN profiles onto GABAergic profiles, which then connect serially to small, presumed Kenyon cell, profiles suggest also possible feedforward inhibitory function.

One identified class of dopaminergic neurons densely arborizes within the locust mushroom body pedunculus and part of the α- and β-lobes (Wendt and Homberg, 1992). Octopamine has also been identified biochemically and histochemically as being present in the mushroom bodies. One identified neuron in the honeybee, the VUMx neuron, may fit in this class (Hammer, 1993). It arborizes within a large subsection of the protocerebrum, including the mushroom body calyces, the antennal lobes, and the lateral lobes.

Olfactory learning and memory and the mushroom body.

In *Drosophila*, combined genetic and behavioral experiments have identified several mutations which are responsible for impaired performance in olfactory learning and memory tasks. Structural mutations cause defective mushroom body morphologies (*mushroom body miniature* and *mushroom bodies deranged*) (Heisenberg, 1985). For biochemical mutations, the mushroom bodies have been shown to be the dominant sites of expression of a number of genes. These genes have been shown to encode several metabolic enzymes in the cAMP pathway in the brain (Livingstone et al., 1984; Nighorn et al., 1991; Han et al., 1992; Levin et al., 1992; Davis, 1993). This pathway includes a cAMP-specific phosphodiesterase (*dunce* gene), a Ca^{2+} calmodulin-responsive adenylyl

cyclase (*rutabaga* gene) and a catalytic subunit of protein kinase A (*DC0* gene). They are expressed by the intrinsic neurons of the mushroom bodies, the Kenyon cells. In combined genetic and behavioral experiments where the expression of these components was reduced or eliminated, the mutant animals showed deficits in associative olfactory learning (Dudai et al., 1976; Dudai, 1988).

Other, non-genetic, manipulations of the mushroom bodies have similar behavioral effects. Chemical ablation of the embryonic mushroom body neuroblasts, which eliminates the intrinsic neurons in the adult, also leads to deficient olfactory learning behavior (deBelle and Heisenberg, 1994). Cooling of the α-lobe or calyx after one-trial conditioning with a small metal probe, a short-term reversible method to suppress activity in the mushroom bodies, results in reduced responses to later test trials (Erber et al., 1980). Conversely, experience by the animal of its environment can cause structural changes in the mushroom body. In flies, sensory (especially olfactory and social) deprivation significantly reduced the number of fibers in the pedunculus (Technau, 1984).

The mushroom body as a site of multimodal processing.

The insect mushroom bodies are probably sites of multimodal sensory processing, in addition to playing a role in olfactory discrimination (Boeckh and Ernst, 1987; Laurent and Davidowitz, 1994) and learning and memory (Erber, 1980; Schürmann, 1974; Hammer and Menzel, 1995; Davis, 1993). Anatomical data shows that while the PNs are the primary source of olfactory input into the calyx, the MB also receives visual afferents (Mobbs, 1982, 1984). Indeed, some insects which lack olfactory antennae also lack calyces, but maintain mushroom bodies which receive input from visual and mechanosensory centers (Strausfeld et al., 1996).

In crickets, cockroaches, and honeybee, physiological experiments show that extrinsic neurons of the MB have multimodal responses (Homberg, 1984; Gronenberg, 1987; Li and Strausfeld, 1997; Schildberger, 1984). Individual extrinsic neurons could

respond to olfactory, visual, mechanosensory (of the antenna or, in the cricket, cercus), and auditory stimuli, and very often responded to two or more modalities. Their responses consisted of phasic or phasotonic excitation or, infrequently, suppression (Schildberger, 1984). The neurons could have different responses (excitatory or inhibitory) to stimuli of different modalities (Homberg, 1984; Gronenberg, 1987; Li and Strausfeld, 1997; Schildberger, 1984), although the response differences within a single modality (i.e., responses to different odorants) were not well characterized. The extrinsic neurons could not be grouped based on their physiological response properties, since each one could respond somewhat differently to the set of stimuli. In addition, long-lasting effects of some stimuli were noted (e.g., prolonged background firing suppression), and these "aftereffects" could influence the responses elicited afterward, even to stimuli of a different modality (Li and Strausfeld, 1997; Schildberger, 1984). Thus, the order or combination of stimuli used could have an effect on responses, indicating an interaction between sensory modalities with a long time constant (i.e., several seconds).

IV. Physiology of the olfactory system.

Olfactory responses of ORNs.

Single neuron recordings.

Olfactory receptor neuron (ORN) responses to odorant stimulation can be measured by means of single- or multi-neuron recordings from the epithelium or antennal sensillae or as field recordings of population activity. The latter type of recording is typically termed electro-olfactogram (EOG) in vertebrates, or electro-antennogram (EAG) in insects. Measuring ORN responses tends to be difficult because of their small size in vertebrates, and their protective encasing in antennal cuticle of insects, in sensilla which are

either deep pits or spiky hair structures. The EOG and EAG are important tools for screening odors that evoke responses, determining basic activity levels, providing evidence on population activity and its distribution, and have even recently been used to assess the functionality of one specific olfactory receptor gene (Zhao et al., 1998). However, single neuron recordings are very important to determine the nature of olfactory responses at the entry point of the system. The response properties of individual ORNs constrain and determine what type of information is available to the rest of the olfactory system.

In vertebrates, the ORN response tuning is widely accepted to be broad. Recordings from single receptor neurons in many species generally reveal responses to multiple odorants, over a range of concentrations (frog: Gesteland et al., 1963; salamander: Getchell and Shepherd, 1978a; frog: Revial et al., 1982; Sicard and Holley, 1984; salamander: Firestein et al., 1993; catfish: Kang and Caprio, 1995).

Recordings from insect sensilla, however, suggest that ORN specificities fall into two categories: first, ORNs with narrow tuning for pheromonal components (Masson and Mustaparta, 1990; Hildebrand and Shepherd, 1997); and second, ORNs with much broader specificities, for plant and other food odors (Sass, 1975; Selzer, 1981; Masson and Friggi, 1974; Mustaparta, 1975), although these generalist ORNs will also often respond to pheromones.

Spatial organization of the peripheral responses.

The salamander mucosa provides a useful system for studying the spatial organization of ORN responses in situ because it is a single flat sheet, rather than a complex folded one like the mammalian epithelium. In the salamander, epithelial responses to single odors were recorded with multiple complementary methods. Punctate epithelial stimulation and mitral cell recordings (Kauer and Moulton, 1974), EOG recordings (Mackay-Sim et al., 1982), and voltage-sensitive dye recordings (Cinelli and

Kauer, 1990; Kent, 1990; Kent and Mozell, 1990) all provided similar results. Responses can be evoked to some degree from sites distributed all over the epithelium, with certain sites defining spatial peaks in the activity. These 'hotspots' are stereotyped across different animals, indicating a genetic or developmental influence (Kauer, 1991). Experimental results from EOGs (Mackay-Sim and Kesteven, 1994) and voltage-sensitive dye recordings (Youngentob et al., 1995) in rat suggest that similarly patterned activity may exist in mammalian olfactory epithelia. Thus there exists both a tendency to map functional responses to certain areas within the receptor sheet, yet the activity remains fairly broadly distributed. These data correspond rather well with the observation, from data acquired by molecular biological techniques in mammals (Ressler et al., 1993; Vassar et al., 1993), that odorant receptor genes are expressed 'randomly' within circumscribed 'zones' in the epithelium.

There is little evidence to suggest a spatial organization of odorant responsivity along insect antennae. In cockroach, while some sensilla types have been correlated with some ORN subtypes (defined by their specificities), the sensilla tend to be intermingled along the length of the antennae (Masson and Mustaparta, 1990; Fujimura et al., 1991; Schaller, 1978). In other insects, such as honeybee, classification of sensilla or ORNs based on physiological response to odorants has been proposed (Vareschi, 1972), and challenged (Akers and Getz, 1992).

Olfactory responses in the central olfactory system of vertebrates.

Olfactory bulb.

Research on olfactory responses in the olfactory central nervous system in vertebrates has focused on the olfactory bulb, a favorable preparation for a number of reasons. The OB is the first olfactory relay site that receives input from the epithelium, and channels olfactory information to higher olfactory sites, and it is a site of significant

convergence of olfactory afferents, before its output projections diverge with respect to the piriform cortex and beyond. The OB has clear structural subdivisions which more or less clearly delineate input, output, and local circuits. The layered structure has led some to propose the bulb as a simple cortical model (Shepherd, 1970). Finally, the olfactory bulb lies directly beneath the cranium on the rostrum, and is thus readily accessible to the experimenter. The next downstream olfactory level, the piriform cortex, lies in the ventral and medial area of the temporal lobe and is therefore less accessible for physiological experimentation.

Mitral cells

Like the olfactory receptor neurons, the mitral cells, the principal olfactory bulb neurons, can respond to more than one odor and are generally broadly tuned (Kauer et al., 1991; Mori and Yoshihari, 1995; Mori et al., 1992; Imamura et al., 1992; Katoh et al., 1993; Yokoi et al., 1995; Wellis et al., 1989; Hamilton and Kauer, 1985). The term 'response' is taken to mean any change in the firing rate or pattern from background, correlated with the stimulus. Any given mitral cell will respond to a variety of single-molecule odors and blends in most species, and these cells are therefore not specialized for single chemical compounds.

Some experiments, however, suggest a link between the chemical structure and ability to evoke a response in a single mitral cell. In the rabbit olfactory bulb, experiments with families of chemically related odors revealed that the neurons will often respond to a cluster of similar odors out of a large group (e.g., *n*-aliphatic aldehydes, *n*-fatty acids, *n*-aliphatic alcohols, etc.)(Mori et al., 1992; Imamura et al., 1992; Katoh et al., 1993). Odors in a cluster differed from one another, for example, by one or two carbons in the carbon chain length, or the addition of a hydroxyl group. While each mitral cell responded to a range of molecules with related chemical structures, different neurons responded with different, but overlapping ranges. Odors were found to evoke inhibitory as well as

excitatory responses. In some cases, odors which evoked strongly excitatory responses were sometimes closely related to those that evoked suppressive responses. Along a stimulus 'axis' of hydrocarbon chain length, for example, excitatory stimuli would be 'flanked' by inhibitory stimuli, while more dissimilar stimuli would cause no response (Yokoi et al., 1995).

Temporal patterning of odor responses.

One obvious characteristic of the patterning of activity in the olfactory bulb neuron responses is the respiratory rhythm (Chaput and Holley, 1980; Chaput, 1986). This rhythmic cycling of inhalation and exhalation (or 'sniffing' cycle) occurs in many small mammals at a frequency of ~4-12 Hz, conventionally called the theta (θ) frequency range. Mitral cell firing is generally modulated with this cycle (Chaput and Holley, 1980; Buonviso et al., 1992) and appears to be the result of phasic stimulation of the olfactory receptors, rather than centrifugal inputs to the olfactory bulb synchronized to respiration (Sobel and Tank, 1993). Here, however, let us consider only temporal patterns of individual neuron odor responses that are unrelated to the respiratory cycle and that are most likely the result of peripheral or olfactory bulb circuitry.

Temporal patterning of individual mitral cells responses involving both excitation and inhibition has been shown in most species investigated (rat, rabbit, salamander, frog) (Hamilton and Kauer, 1989; Harrison and Scott, 1986; Meredith, 1986; Wellis et al., 1989). While responses to some odors are solely excitatory or solely inhibitory, a significant fraction of responses are more complex, involving multiphasic firing patterns that often combine excitation and inhibition. Most of these patterns are on a time scale of tens to hundreds of milliseconds (although some last up to several seconds), which can subdivide the stimulus duration or outlast it. Different patterns are evoked in individual neurons for different odors (and are therefore odor-specific). However, different patterns can be evoked by the same odor in different neurons in the same animal (and therefore are

odor- *and* neuron-specific). Intracellular recordings verify that firing suppression is often correlated with hyperpolarizing inhibition, suggesting that the inhibitory interneurons play a role in shaping the mitral cells response patterns (Hamilton and Kauer, 1989). Odors thus evoke in the olfactory bulb neurons patterns of activity that have both spatial (or neuron identity) and temporal characteristics.

Several studies have revealed that the temporal patterns may vary with concentration. Changes in concentration can not only alter the strength of excitation and inhibition (simple quantitative effects), but can sometimes have qualitative effects, shifting, for example, an inhibitory-excitatory pattern to an excitatory-inhibitory pattern (Meredith, 1986; Wellis et al., 1989). The changes, however, are gradual over gradual concentration changes (Wellis et al., 1989), unlike the immediate and complete shift that occurs when an odor is changed (Meredith, 1986; Wellis et al., 1989). Also, comparison of the concentration-response profiles for two odors in the same neuron reveals that the profiles can be odor-specific: with some pairs of odors A and B, at no concentration did the response for odor A look like the response for odor B (Wellis et al., 1989). These effects may be due to changes in odor-evoked activity at the periphery, since the relative binding of the ligand with the receptor proteins themselves will change with concentration. Such effects of concentration on the temporal pattern will be important to understand for a theory of olfactory coding based on spatiotemporal activity. It will be interesting to determine whether changes in temporal patterning are correlated with changes in odor perception, which is itself not entirely concentration invariant.

Inhibitory interneurons: granule and periglomerular cells.

Physiological and pharmacological studies of olfactory bulb neurons using electrical stimulation of the input tract (olfactory nerve) and the output tract (LOT) have strongly suggested that the granule interneurons provide inhibitory output (see below; Getchell and Shepherd, 1975a,b; Hamilton and Kauer, 1988; Isaacson and Strowbridge,

1998; Jahr and Nicoll, 1982; Mori and Takagi, 1978; Nowycky et al., 1981; Rall et al., 1966; Shepherd, 1971). Computational approaches (Rall et al., 1966; Rall and Shepherd, 1968; Shepherd and Brayton, 1979) predicted and physiological experimental evidence supported (Jahr and Nicoll, 1982) the idea that dendrodendritic synapses between granule cells and mitral cells mediate the inhibitory effects witnessed with antidromic (LOT) electrical stimulation.

Recently, the responses of the local olfactory bulb interneurons to odor stimulation have been recorded in rat (Wellis and Scott, 1990). Granule cells have odor responses which generally consist of simple excitatory spiking responses or non-spiking, depolarizing responses. These responses are reliable and consistent for any given odor. Few granule cells were shown to have inhibitory responses, and in general their responses are much less than those reported for mitral cells, i.e., they are not usually multiphasic. All the granule cells recorded were capable of firing spikes when tested with an intracellular depolarizing pulse (Wellis and Scott, 1990). The spiking and nonspiking responses may indicate subtypes of granule cells, since response type correlates with position in the layer. However, it has been shown that an action potential is not necessary for synaptic transmission at the dendrodendritic synapse (Jahr and Nicoll, 1982; Shepherd and Brayton, 1979), leaving open the question of the role of granule cell spiking responses in olfactory processing. Granule cells can respond to more than one odor, but the breadth of their odor "tuning" remains to be tested (Wellis and Scott, 1990).

Neurons in the glomerular level are also capable of producing spiking responses to odor stimulation. Identified periglomerular cells have been reported to respond to multiple odors (in limited testing) with simple bursting excitatory responses, less complex than those of granule cells (Wellis and Scott, 1990). The periglomerular neurons appear to respond to a wider spatial array of afferent input than would evoke responses in either mitral or granule cells, consistent with the lateral spread of their dendrites across multiple glomeruli. Several other cell types of the glomerular layer include the short-axon neurons

(Getchel and Shepherd, 1975b; Schneider and Macrides, 1978) and the putative juxtaglomerular cells (Bufler et al., 1992a), which may differ in their morphological and physiological characteristics. It is unknown whether granule and periglomerular cells, or the other putative inhibitory interneurons, have functionally different effects on the mitral cell activity and integration of olfactory information in the bulb.

Synaptic interactions.

When mitral cells are antidromically activated (a common way to test for mitral cell impalement), they undergo a long-lasting hyperpolarization following an initial action potential (Phillips et al., 1963). The source of this hyperpolarization is reciprocal dendrodendritic synapses between mitral and granule cells, so that when a mitral cell is activated, it excites a granule cell via NMDA and non-NMDA glutamate receptors, which in turn inhibits the mitral cell (Isaacson and Strowbridge, 1998; Jahr and Nicoll, 1980, 1982a,b; Nowycky et al., 1981; Wellis and Kauer, 1993, 1994). In an early success of computational neurobiology, Rall and Shepherd developed a model of the bulb which predicted the existence of dendrodendritic synapses between mitral cells and granule cells (Rall et al. 1966; Rall and Shepherd, 1968); concurrently, dendrodendritic synapses were found anatomically (Rall et al. 1966; Reese and Brightman, 1965; Reese, 1966; Price and Powell, 1970). By blocking action potentials with tetrodotoxin, it was demonstrated that the inhibition does not rely on mitral cell axon collatorals, and that it was indeed likely dendrodendritic (Jahr and Nicoll, 1982). A calcium spike which persists without Na^+ channel activation is sufficient to cause reciprocal inhibition. The slow inhibition appears to result from a summation of fast IPSPs mediated by a $GABA_A$-receptor (Isaacson and Strowbridge, 1998). Thus, the granule cells are thought to provide self-inhibition of mitral cells via dendrodendritic synapses.

The granule cells may also be responsible for lateral inhibition. For example, inhibitory potentials and firing suppression is evident in mitral cell odor and electrical

stimulation responses in which no previous depolarization or action potential occurred (thus ruling out self-inhibition) (Hamilton and Kauer, 1985; Kauer and Hamilton, 1987). Pharmacological manipulations during odor stimulation also appear to provide support for a role by granule cells in lateral inhibitory synaptic effects in mitral cell odor responses. As described above, Mori and coworkers found that in mitral cells, similar odorants that evoke excitatory responses are often 'flanked' by less similar odorants that evoke inhibitory (suppressive) responses. The suppressive effects of odorants can be blocked by blocking synaptic transmission in the layer containing the mitral and granule cell dendrites, either directly by GABA antagonists (blocking the granule-to-mitral cell synapse), or indirectly by glutamate antagonists (blocking the mitral-to-granule cell synapse) (Yokoi et al., 1995).

Granule cell inhibition, then, may help shape mitral cell responses and sharpen their tuning. It has been suggested that the granule cells contribute to a center-surround generating mechanism, by analogy with the visual system (see Mori and Yoshihara, 1995). The function of the periglomerular cells and other putative inhibitory interneurons in olfactory bulb odor processing has yet to be determined.

Spatial organization of olfactory bulb activity.

Physiological recordings from the olfactory bulb suggested a broad distribution of activity in response to olfactory stimuli (Adrian, 1942; Viana di Prisco and Freeman, 1985). Molecular biological experiments, however, demonstrated that olfactory receptor neurons project to the olfactory bulb with a precise organization of axons deriving from ORNs expressing identical receptor gene types terminating in single (or pairs of) glomeruli (Ressler et al., 1993; Vassar et al., 1993; Mombaerts et al., 1996). Functional mapping with numerous methods (2-deoxyglucose uptake, c-fos expression, voltage-sensitive and calcium dye optical imaging, electrophysiology) suggests that there is a spatial organization of the olfactory bulb responses to odors (Kauer, 1991; Leveteau and MacLeod, 1966; Lancet et al., 1982; Guthrie et al., 1993). Distinct groups of glomeruli are activated by

different odorants, suggesting that glomeruli are not only histological, but functional units. Optical imaging of the entire (or large sections of) olfactory bulb showed that while activity is evoked over broad areas, and encompassed multiple glomeruli, the patterns were odor-specific and distinct but overlapping for different odors (Cinelli et al., 1995b; Friedrich and Korsching, 1997). These results suggest that a combinatorial, distributed code exists in the olfactory bulb involving a large number of neurons.

Interpretations of these results reveals a tension between the desire to demonstrate a neat mapping of olfactory space (glomeruli form functional units which sort out the molecular 'picture' and represent features, by analogy to the visual system; see Shepherd, 1991) and the distributed nature of the activity (reflecting a high degree of parallel processing and the coarse nature of odor encoding at the single neuron level; see Kauer, 1991). In behavioral experiments, lesions of glomeruli known to respond to a particular odor did not impair the detection or discrimination of that odor (Slotnick et al., 1997). This result suggests that the bulb does not depend on encoding any given odor with activity in single glomeruli, and that the distributed nature of olfactory information in the bulb may be important. However, more research is needed to determine the precise role of the well-ordered spatial patterns in the bulb in olfactory information processing.

There are three basic differences between the ORN odor responses and olfactory bulb responses. First, while individual ORNs are broadly tuned and show mostly monotonic, excitatory responses, individual OB neurons are broadly tuned, and show more complex responses. These complex responses are sometimes elaborate but consistent temporal patterns often consisting of both excitation and inhibition. Second, there is a qualitative difference in the spatial topography of odor responsivity in the epithelium and bulb. In the epithelium, single odorants evoke responses across odor-specific and stereotyped, but widespread, areas. In the bulb, there appear to be more distinct spatial patterns, corresponding to overlapping combinations of odor-activated glomeruli, although the activity is still broadly distributed across many neurons. Third, the olfactory bulb (and

higher olfactory areas) is distinguished by prominent oscillatory population activity, an indication of neural synchronization, which does not appear in the periphery of most animals studied (with an exception with amphibians: Hamilton and Kauer, 1989).

Piriform cortex

Comparatively few studies have been done of the piriform cortex compared to the olfactory bulb, probably because of its relative inaccessibility. Thus, few recordings from single units have been made in the piriform cortex. One study (Tanabe et al., 1975) investigated the odor responses in awake monkeys and found that neurons there responded to multiple odors (averaging three out of a panel of eight) and most often with excitatory responses, although inhibitory and multiphasic responses occurred. One interesting result was found in comparisons of odor responses in the olfactory bulb, piriform cortex and frontal olfactory cortex recordings. The physiological responses of neurons to similar odors (chemically related 'camphor'-type odors) were progressively more distinct (more discriminable to an observer) as one proceeded from the bulb to the olfactory frontal cortex.

The piriform lacks such obvious modules like the OB glomeruli, and anatomical tracing experiments showed that the projection patterns of the LOT afferents is diffuse and only weakly topographic, unlike the projection patterns from the epithelia to the bulb. Functionally, as well, there is no clear topography in the piriform cortex, although few physiological experiments involving natural odor stimulation have been reported (Haberly, 1985). Deoxyglucose activity labelling of the piriform cortex during olfactory stimulation, for example, shows only a broad patterning across the area. These results must also consider the associative connectivity within the olfactory cortex.

Oscillations and synchronization in vertebrate olfaction

Electroencephalogram and local field potentials in the brain.

The invention of the electroencephalographic recording (EEG) by Berger in the late 1920's led to the discovery of brain waves: oscillatory potentials so widespread and coherent that they can be recorded from the scalp, establishing the generation of oscillatory activity in the brain (Berger, 1929). Scalp EEGs and other oscillatory extracellular potentials are thought to be the result of summed synchronous neural activity (usually synaptic potentials) of a large population of neurons. Numerous studies have attempted to relate these brain waves with functional states (such as sleep-wake cycles or consciousness) and stimulus-evoked oscillatory activity have been used to map physiological function to localized sites in the human brain (see reviews in Creutzfeldt, 1995; Steriade et al., 1990). Direct physiological measurements from the brain tissue of animals in the last 50 years have provided a great deal of evidence pertaining to the nature of the neural activity underlying the (extracranial) EEG, leading to an understanding of different types of oscillations (often divided by frequency range) in normal function and pathological activity (characterized by 'seizures'). In this section we focus exclusively on oscillatory synchronization in normal brain function.

Odor-evoked oscillations in the vertebrate olfactory system.

Early evidence for oscillations in olfactory brain structures comes from the experiments of Lord Adrian in the mammalian olfactory bulb and piriform cortex (Adrian 1942, 1950, 1950, 1954; also Ottoson, 1959a). Field electrodes in the olfactory bulb recorded an "induced wave" in a frequency range of 40-80 Hz (conventionally referred to

as the γ range, or fast) at each inhalation during the presence of an odor stimulus, distinct from the intrinsic activity or the θ-frequency oscillations which are related to inhalation of unodorized air (sniffing cycle). These fast oscillations are present at the level of the olfactory bulb and piriform cortex in a variety of mammals, such as hedgehog (Adrian 1942), rabbit (Adrian 1950; Bressler and Freeman 1980), cat (Freeman, 1960; Bressler and Freeman 1980), rat (Bressler and Freeman 1980), dog (Domino and Ueki, 1960), monkey (Domino and Ueki, 1960), as well as humans (Hughes et al. 1969). It is similar to rhythmic activity noted in the olfactory systems of non-mammalian vertebrates such as fish and frog (Adrian and Ludwig, 1938; Bressler and Freeman, 1980; Ottoson, 1959b; Libet and Gerard, 1939). Mitral cell firing activity has been shown to be phase-locked to the EEG oscillatory cycle (Freeman, 1975; Eeckman and Freeman, 1990).

Fast oscillations in the piriform cortex are highly correlated with those simultaneously recorded in the olfactory bulb (Bressler, 1987a) and are likely partially driven by input from the bulb (Bressler, 1987b). If one severs the lateral olfactory tract (Freeman, 1968; Bressler, 1987b) or blocks activity in the olfactory bulb (Gray and Skinner, 1988a), the fast oscillations are abolished in the piriform cortex, but persist in the bulb in response to olfactory stimulation. This suggests that the bulb is capable of generating oscillatory activity in response to an olfactory stimulus (Freeman, 1975). However, ample evidence suggests that the piriform cortex also has oscillatory dynamics independent of oscillatory bulbar input: driving the LOT with a strong stimulus produces a damped oscillatory field potential in the piriform cortex (Freeman, 1968a; Freeman, 1968b; Bressler and Freeman, 1980; Ketchum and Haberly, 1988). Modelling studies of the piriform cortex support this conclusion (Wilson and Bower, 1992). It thus appears that the functional architecture of the olfactory cortex is properly 'tuned' to accept oscillatory input from the bulb.

How are the fast oscillations related to the encoding of odors? Freeman and colleagues studied extensively the EEG in mammalian olfactory bulb with an array of

electrodes which could record from ~20% of the bulbar surface (Freeman, 1975). These experiments indicated a highly distributed pattern of activity, involving nearly the entire bulb for any odor stimulus. A high degree of coherence in the phase of activity across the bulb was found, but there was no odor specificity in the macroscopic field pattern, suggesting that neither the phase nor spatial aspect of the pattern represented the odor. Similarly, the frequency of the induced wave was variable, but failed to correlate with odor identity (Freeman, 1978; Freeman and Schneider, 1982). Only when the animals were stimulated with odorants within a behaviorally relevant context (classical discriminative conditioning) were patterns significantly different from control induced activity (odorants passively presented) (Viana di Prisco and Freeman, 1985; Freeman and Grajski, 1987). These patterns evolved over the course of training, indicating context- and experience-dependent activity changes and were related to the expectations of the animal. These observations led Freeman to hypothesize that the representation of an odor in the OB is stable only when the odor has acquired a particular contextual meaning.

The rhythmic activity in the olfactory bulb is believed to be the result of reciprocal dendrodendritic circuits between mitral cells and the granule cells and their dynamics (Freeman, 1964; Shepherd and Brayton, 1979), controlling the mitral cell output in response to a sustained input. This hypothesis, however, has never been directly tested. The same mechanisms have been suggested to be involved in both self-inhibition and lateral inhibition (Shepherd and Brayton, 1979). Blocking inhibitory mechanisms in the olfactory bulb appears to alter the firing rates and tuning of odor responses in the principal neurons (Yokoi et al., 1995). However, it is not known whether mechanisms responsible for generating oscillations in the olfactory bulb are distinct from those that help define mitral cell tuning and responses. If the mechanisms responsible for the oscillations in the bulb are also involved in other functions, it is possible that any rhythmicity generated may be an epiphenomenon, that is, a result of other factors which neither adds nor detracts from

information processing. Further details about the exact mechanisms and empirical tests of any hypotheses are needed, and will be addressed here, using the insect model system.

Olfactory responses in the central olfactory system of insects: the locust as a model system.

Physiological recordings have been made from the antennal lobe neurons of a number of insect species (e.g., honeybee, cockroach, moth, locust). Odor responsivity of the antennal lobe neurons allows us to broadly categorize them into those which respond to conspecific sex pheromones ('specialist') and those which respond to a broad range of food and other odors ('generalist'). The specialist neurons typically arborize in the macroglomerular complex, and are highly selective for pheromonal components, or specific blends of pheromonal components. Generalist neurons arborize in the 'ordinary' (non-macroglomerular) glomeruli, and can respond to a broad range of odors, sometimes including the pheromone components. In the following description of insect central olfactory physiology, we will focus primarily on non-pheromonal data from the locust, which appears to lack a sexually dimorphic pheromonal specialist system.

Locust antennal lobe neuron odor responses.

The projection neurons (PNs) in the locust are tuned, like other first order neurons in vertebrates and insects, and respond selectively to a range of odors. The PNs are also "tuned" in that different odors can evoke distinct slow temporal patterns (Laurent and Davidowitz 1994; Laurent et al., 1996). Temporal patterning of activity has been observed in several other insects (honeybee, Stopfer et al., 1997; cockroach, Burrows et al., 1982; Kanzaki et al., 1989; moth, Christensen et al., 1998). In locusts, PN responses to odors consist of temporal patterns that may be excitatory, inhibitory, or, as is often the case,

consist of several sequential epochs of excitation and inhibition, where each epoch could last for 100-1000 ms. We designate these patterns as "slow" temporal patterns, to distinguish them from the fast oscillatory patterning that occurs on a 30-50 millisecond time scale (see below).

Any given odor, however, does not elicit the same pattern from every neuron; that is, cherry, for example, may elicit an transient excitatory response in one PN, but a complex multiphasic response in another PN (within the same animal). Likewise, any given PN can show very different slow temporal patterns for different odors (i.e., excitatory for cherry, but inhibitory for apple). The response pattern by a PN for any one odor, however, is reliable over many trials (Laurent and Davidowitz, 1994; Laurent et al., 1996; MacLeod and Laurent, 1996). From these data, we propose a qualitative description of the ensemble response to stimulation with one odorant: an odor elicits a response from only a subset of PNs (estimated to be ~10% of the population), and each PN only fires action potentials during certain epochs over the duration of the stimulus. Thus the ensemble of neurons that are firing roughly simultaneously (within a ~100ms window) changes over time in an odor-specific manner. A different odor would lead to activation of a different, but overlapping, ensemble of neurons with its own temporal patterning. Odor representation, then, may rely on a specific "spatial" pattern (i.e., which of the PNs are activated) and a specific temporal pattern (i.e., determined by when these neurons are activated). The temporal pattern of the individual PNs, indeed, define an evolving "spatial" pattern.

The other prominent neuronal type found in the AL are the local interneurons (LNs). These neurons can be physiologically differentiated from PNs in locust because they are nonspiking, i.e., they do not produce overshooting, Na^+-mediated action potentials. Instead, their activity in response to odors generally consists of slow depolarizing potentials and the membrane potential oscillations described below (Laurent and Davidowitz, 1994). In many other insects, the local interneurons appear to be spiking

(Anton and Hansson, 1994; Christensen et al., 1993; Hoskins et al., 1986; Fonta et al., 1993; Sun et al., 1993).

Oscillations and synchronization characterize the insect olfactory system.

Synchronized oscillatory neural activity in the insect olfactory system was found first in the locust mushroom body (Laurent and Naraghi, 1994) but has since been found in the mushroom bodies of the honeybee, bumble bee, cockroach, and wasp (Stopfer et al., 1997; Stopfer and Laurent, 1995) and in the antennal lobe of a moth (Heinbockel et al., 1998).

Oscillations in the mushroom body.

Odor-evoked oscillations can be observed in the mushroom body of the locust by placing a low impedance electrode in the dorsal calyx neuropil and recording the local field potential (LFP)(Laurent and Naraghi, 1994). An odor puffed onto the intact ipsilateral antenna evokes characteristic large-amplitude (0.2-1.0 mV) oscillations that persist over the duration of the stimulus and dissipate immediately after the offset of the stimulus (Laurent and Davidowitz, 1994; Laurent and Naraghi, 1994). Spectral analysis of these oscillations shows a distinct peak in the 20-30 Hz frequency range. An air puff has no effect on the LFP other than a slow negativity and does not produce oscillations or an increase in the spectral power in the 20-30 Hz band (Laurent and Naraghi, 1994). Like the results reported in OB (Freeman, 1978; Freeman and Schneider, 1982), the odor-evoked oscillations do not "code" for odor identity either by their spatial pattern (i.e., they are coherent across the MB) or by frequency characteristics (i.e., all odors elicit oscillations of the same 20-30 Hz frequency) (Laurent and Davidowitz, 1994; Laurent and Naraghi, 1994). The "envelope" of the oscillations varies nearly as little across different odors as across trials of the same odor, and therefore provides little information to an observer about

stimulus identity. These odor-evoked LFP oscillations should be understood as a macroscopic phenomenon, not something retrievable by the nervous system, but rather simply as a measurement of the underlying synchronized, rhythmic population activity.

Kenyon cells.

While the morphology of the intrinsic neurons of the MB, the Kenyon cells (KCs), has been known for over a century (Kenyon, 1896; Pearson, 1971; Schürmann, 1974; Mobbs, 1982), their physiology has only been recently described. Intracellular recordings from the KCs reveal that they respond to odor stimulation with depolarizing synaptic events which are rhythmic, resulting in an oscillation around resting potential (Laurent and Naraghi, 1994). The amplitude of these depolarizing events can be amplified by active, voltage-dependent membrane properties. Kenyon cells rarely produce strong, excitatory responses; rather, their firing is often suppressed during the oscillatory potentials. If a KC does spike, the action potential usually occurs at most on every other cycle of the oscillation (Laurent and Naraghi, 1994). The KC oscillatory membrane potentials and the spikes triggered during depolarization are phase-locked to the LFP recorded simultaneously in the MB with a different electrode. The depolarizing potentials and action potentials occur during the descending phase of the LFP cycle (after the peak, before the trough).

Antennal lobe neuron odor responses also contain oscillations.

Odors presented to the intact antenna cause odor-specific responses in the PNs with two temporal components. First, the PNs respond with slow temporal patterns as described above. Second, PN responses contain fast membrane potential oscillations in the 20-30 Hz bandwidth.

During odor stimulation, the membrane potential of the PNs often show rhythmic synaptic activity, consisting of fast alternating EPSPs and IPSPs (Laurent and Davidowitz,

1994). These membrane potential oscillations regulate the firing of the PNs such that they fire in synchrony with other PNs. When a PN fires a spike during an odor response (during the depolarizing phase of the EPSP), the action potential is often tightly (within 0-12 millisecond jitter) phase-locked to the LFP oscillation. These spikes that are phase-locked occur during the rising phase of the LFP cycle, as measured in our experiments (after the trough, before the peak). It should be noted that not all spikes evoked during an odor response are necessarily phase-locked to the field potential, but instead occur at random times relative to the oscillation (Laurent et al., 1996). The transient phase-locking of a PN, however, occurs reliably during an odor-specific time epoch during the response. All PNs show the same phase-locked relationship with the LFP regardless of odor (Laurent and Davidowitz, 1994; Wehr and Laurent, 1996). This is true whether one records sequentially from many PNs, or simultaneously from two or more PNs (Laurent et al., 1996). Thus any PNs who are firing and phase-locked to the LFP oscillation must, by default, be phase-locked, or synchronized, to one another.

LNs, like the PNs, show odor responses that contain membrane potential oscillations resulting from alternating fast EPSPs and IPSPs and generally superimposed on a sustained or phasotonic depolarization. The LN responses are also phase-locked to MB LFP oscillations but with a 90° phase-shift relative to the PN spikes (Laurent and Davidowitz, 1994).

Odors are encoded by synchronous neural ensembles.

The field potential oscillation is thus a result of synchronous firing of PNs which provide the input to the MB. The continuous nature of the LFP oscillation must be caused by the sequential and overlapping activity of different groups of PNs firing at any given time, since individual PNs only fire for certain odor-specific periods. While it can be shown that PNs only synchronize with the other PNs for a subset of spikes, the time at which it synchronizes is reliably evoked and odor-specific. We thus arrive at a hypothesis

of odor encoding via a sequential activation of synchronously firing neurons, where each cycle of the oscillation is caused by a slightly different, and continuously evolving, group of neurons (Laurent et al., 1996; Wehr and Laurent, 1996). Odor representation may be encoded by a spatiotemporal activation pattern on a very fine (~50ms) time scale (Wehr, 1999; Wehr and Laurent, 1996).

Olfactory models.

We will discuss here several explicit models created to define and attempt to solve problems of olfactory information processing, or to reproduce physiological characteristics of olfactory areas. The olfactory system has been a leading topic in the area of computational neuroscience, thanks in part to the simplifying concepts of canonical circuit (Shepherd, 1990) and the idea of the olfactory bulb and piriform cortex as simple cortical systems, the application of cable theory (Rall et al., 1966), and the suitability of associative networks to olfactory problems (Hopfield and Tank, 1986; Hopfield, 1991). A number of models specifically investigate the role of oscillations and synchronization (Hopfield, 1995; Li and Hopfield, 1989; Li, 1990; Linster et al., 1993; Wilson and Bower, 1992; Wang et al., 1991).

Most approaches to higher olfactory modelling focus on some type of pattern recognition. One pattern is simply that of the external stimulus (the combination of odorant molecules and/or their steric/chemical qualities, or 'odotopes' (Shepherd, 1991)). The stimulus is generally thought to be directly translated into a second pattern, that of the ORNs' neural activity in response to the stimulus. There is some debate as to whether this translation is linear; direct experimental tests caution against an assumption of linearity (the activity resulting from a mixture of two odors is the sum of the activity evoked by each odor alone), but it is often invoked for simplicity at the level of the periphery.

Recognition, however, implies that there is something to be 'recognized', e.g., a previously learned pattern. Simple pattern completion will be sufficient when the organism must recall a singular odor (possibly implemented with associative networks, e.g., Hopfield, 1982). If new odors are encountered, however, some sort of learning algorithm must be implemented. Several models investigate ways of doing this by tagging a novel odor as 'unknown' (e.g., Getz, 1991) and invoking synaptic plasticity models (Hasselmo et al., 1990).

Detecting complex odor blends in a noisy background and major and minor component ratios, however, are tasks that call for feature detection, or object segmentation. Several ways of approaching such tasks involve fluctuation analysis (Hopfield, 1991), segmentation by synchronization (Wang et al., 1991) and adaptation (Hopfield, 1996; Li , 1990) to separate the known from the unknown.

Several models have investigated the biophysical properties of neurons of the olfactory bulb, focusing on single neurons (Bhalla and Bower, 1993) or synaptic interactions between subcompartments of single neurons (Rall et al., 1966; Rall and Shepherd, 1968; Shepherd and Brayton, 1979). One of the first explicit models using Rall's cable theory and compartmental modelling techniques was an olfactory model, which included an investigation of the dendrodendritic interactions of mitral and granule cells (Rall et al., 1966; Rall and Shepherd, 1968).

We will discuss in greater detail a few of these models which are pertinent to the generation and computation significance of the temporal patterning of activity in the olfactory systems of vertebrates and insects.

Network models of olfactory bulb and antennal lobe.

The model of Rall et al. (1968) was primarily designed to explain olfactory bulb field potential recordings in electrical stimulation experiments. This model was a moderately realistic network model, which included a population of neurons (scaled

down), compartmental modelling of individual neurons, and a calculation of the expected extracellular potentials. This model examined the effect of dendrodendritic interactions between mitral and granule cells, as tested with anatomical (Rall et al., 1966) and physiological (Jahr and Nicoll, 1982) experiments.

Several models have sought to reproduce, and thus understand, the origin of the temporal patterning of odorant responses (see section above). Meredith (1992) provides a simplified model to explain the non-monotonic changes in the firing rate response to different odor intensities found in physiological experiments (Meredith, 1986; Wellis et al., 1989). The patterns are a result of the spatial mapping of odor responses and a convergence of steady-state excitation and inhibition, whose net effect is concentration-dependent.

White et al. (1992) created a more detailed biophysical model of the olfactory bulb, which sought to reproduce the range of intracellular membrane potential behaviors of mitral, granule and periglomerular cells. The change in response patterns of individual model neurons over odor stimulus (concentration) intensity were similar to those that occur *in vivo*, including a brief hyperpolarization that often occurred prior to an excitation-inhibition pattern frequently seen at moderate intensities. Long-lasting depolarizations were seen in the periglomerular and granule cells, as observed in physiological recordings (Wellis et al., 1990).

A mathematical model of the olfactory bulb investigated the production of γ-frequency oscillatory activity in response to non-oscillatory inputs (Li and Hopfield, 1989). Simulations reproduced coherent oscillatory activity whose exact output pattern across the model mitral cells activity allowed classification of the input pattern. The model repoduced several known features of the oscillatory activity in the olfactory bulb, such as the phase-relationship between mitral and granule cells, EEG frequency coherence, non-zero phase gradient across the bulb, the superposition of the high-frequency oscillation onto the low freqency sniff cycle (Freeman, 1975).

An explicit model of the insect macroglomerular complex was investigated using a small network of neurons (Linster et al., 1993). This model also reproduced the range of projection neuron response patterns reported during odor stimulations. These responses could be used to discriminate the ratio of input odors, representing the major and minor components of pheromones. Another, more simplified model investigated the possible function of oscillatory activity in the antennal lobe (Linster et al., 1994). In this system, the reciprocal connections between the two neuronal populations (projection and local interneurons, each lumped into a single variable) resulted in a resting oscillatory activity. Upon stimulation with the preferred ratio of inputs (major and minor pheromonal components), the neuronal activity moved into a regime in which the neurons fired in an oscillatory fashion. The improper ratio saturated or silenced the activity.

While these results successfully reproduce some of the features of odor processing in mammalian olfactory bulbs and insect antennal lobes, further work needs to be done in light of new data, such as the precise projections of afferent inputs, updated connectivity between constituent neuronal types, and a better understanding of the synaptic effects of these connections. As models of olfactory bulb and antennal lobe function progress, it will be interesting to observe whether the models will converge onto a common functional architecture.

Network models of piriform cortex .

In his landmark report of the circuitry of the piriform cortex, Haberly (1985) suggested that the features of the piriform cortex organization might subserve learning and memory, and indicated that it might provide a model for associative, or content-addressable, memory networks (Haberly, 1985; Haberly and Bower, 1991). Two features that suggest associative memory functions are the distributed, nearly non-topographic nature of the sensory representation (Tanabe et al., 1975; Haberly and Price, 1977), and the diffuse, extensive distribution of local excitatory interactions (Haberly, 1985). Networks

implementing this type of memory function has been shown to be effective in pattern completion from a noisy template (Hopfield, 1982). Modifications of such a nework to utilize oscillatory attractor states may represent a solution to odor object segmentation (Wang et al., 1991).

A large-scale model of the piriform cortex (Wilson and Bower, 1992) constrained by physiological and anatomical considerations successfully reproduced one of the known features of olfactory cortex: the oscillatory activity resulting from electrical and odor stimuli (Freeman, 1968b; Bressler and Freeman, 1980). Most intriguingly, the large-scale patterning of activity suggested a convergence of afferent and associative (recurrent) information, due to an oscillatory looping of activity (Wilson and Bower, 1992). With such convergence one could imagine a type of 'matching' process, comparing information on successive cycles or possibly comparing input patterns with learned patterns.

In summary, the results of these modelling studies suggest that the temporal patterning of odor responses in the olfactory bulb and antennal lobe, and the oscillatory activity generated in the olfactory bulb and piriform cortex, may be computationally significant for the recognition of odor identity and olfactory memory function.

V. Synchronization in other systems.

Oscillatory synchronization of neural activity is readily observable in the olfactory systems of many species. Neural synchronization is prevalent in many other areas of the brain as well, such as the visual, auditory, somatosensory and motor cortex, the thalamus, and the hippocampus. Understanding the role that synchronization plays in olfactory systems may help us understand similar phenomena in other parts of the nervous system.

Oscillations and synchronization.

Oscillatory population responses, such as those measured in local field potentials, are generally thought to reflect underlying oscillatory, synchronized firing of a large number of neurons. However, oscillations and synchronization are separate phenomena which, in principle, need not both be present. Neurons may synchronize their firing in a non-oscillatory pattern of activity; such synchrony could therefore go undetected in spectral analyses of field activity. Similarly, an individual neuron may fire in an oscillatory fashion but not in synchrony with other neurons.

Synchronization and perception.

The objects we perceive around us are defined by many different properties (contours, color, motion). Identifying these objects, and separating them from one another or from the background, requires information about these properties and specificially how they are combined, or linked. Neural synchronization has been proposed as a solution to this problem of perceptual binding (Milner, 1974; von der Malsburg, 1981). In this scheme, neurons that code for the various properties of the same object fire in synchrony. Synchronization, then, may be a neural tag which can be used to flexibly and dynamically link features. Others have proposed that temporal correlations among neurons might allow the selection object representations for further processing (Neibuhr and Koch, 1994).

Neural synchronization and stimulus binding in the visual system.

The experimental evidence for such temporal binding mechanisms has been best established in the mammalian visual system. Temporally correlated discharges had been long investigated, initially focused mainly on determining neuronal connectivity between simultaneously recorded neurons. A major impetus to the study of the temporal correlation hypothesis occurred with the demonstration of stimulus-dependent, synchronized, oscillatory (~40 Hz) discharges across spatially adjacent neurons in cat

visual cortex (Eckhorn et al., 1988; Gray and Singer, 1989; later shown in monkey; Livingstone, 1991; Eckhorn et al., 1993). Presentation of a preferred stimulus results in the occurance of synchronizated burst discharges with an interval of 15-30 ms in the multiunit recordings of closely spaced neurons. These bursts are correlated with γ-band oscillations in the local field potential. Neurons located in different cortical columns can synchronize if these neurons are tuned to similar stimulus characteristics (e.g., orientation) (Gray et al., 1989; Engel et al., 1990).

Neuron synchronization in the visual system was shown to be dependent on global stimulus parameters (Eckhorn et al., 1988; Gray et al., 1989, Engel et al., 1991a,c). Consider a stimulus that consists of a single, continuous bar. Passing this stimulus over non-overlapping and collinear receptive fields of two neurons with similar orientation preferences caused a response in each neuron and synchronization between the activity of these neurons. However, stimulation that consisted of two separate bars moving in opposite directions over the same receptive fields produces nearly identical spiking responses in the neurons, but their activity did not synchronize. These data suggested that synchronization between neurons occurs when neurons encode stimuli that have a unified, coherent set of properties, linking them into what can be called an object. The temporal correlations of feature specific responses of neurons within a brain area could then, in principle, be used as a mechanism for segmentation and binding of objects.

Further experiments demonstrated inter-areal and inter-hemispheric synchronization (Eckhorn et al., 1988; Engel et al., 1991a,b). This long-distance synchronization was also characterized by dependence on global stimulus parameters and occurred between cells that had similar receptive fields and orientation selectivity. Most remarkably, neurons showed near-zero time lags despite the large interhemispheric distances between them. This correlation depended on an intact corpus callosum (Engel et al., 1991b). These observations agree with the prediction of the temporal coding hypthesis that the cell ensemble encoding for an object should be distributed across different areas.

If neural synchronization is involved in the perceptual binding and segmentation (or grouping), then synchronous activity should be correlated with the perceptual state of the animal. Binocular rivalry, in which one of a pair of incongruous images presented to each eye is suppressed while the other is perceived, provides a convenient case for testing the mechanisms involved in dynamic perception (Fries et al., 1997). During dichoptic stimulation, there was an increase in cortical synchronization in neurons which were responsive to the perceived stimulus (relative to monocular stimulation) while a decrease occured in neurons which were responsive to the suppressed stimulus. The degree of synchronization, thus, was correlated with perception.

Mechanisms of neural synchronization in the visual system.

While studies of neocortical synchronization have correlated coherent firing with stimulus or behavioral parameters, the mechanisms underlying it are not well understood. Cortical synchronization may depend on thalamic drive or on intracortical mechanisms. Observations of neural synchronization have also been made in the thalamic networks at frequencies similar to those found in cortex (Ghose and Freeman, 1992; Steriade et al., 1991, 1996b). Coherenct fast oscillatory activity could be observed between thalamocortical and corticothalamic neurons which are reciprocally connected (Steriade et al., 1996b). The synchronization within thalamic networks appears to be at least partially dependent on cortical feedback (Sillito et al., 1994).

Intracortical network dynamics are likely to play a role in generating synchronized neural activity between cortical neurons. Theoretical studies suggest that interactions between excitatory and inhibitory neurons are likely to underlie the oscillatory mechanisms which could serve to coordinate the activity of many neurons. Several recent models of oscillatory synchronization involve some type of inhibitory network interaction (Lytton & Sejnowski, 1991; Bush and Sejnowski, 1996); in fact, mutually inhibitory networks may be more effective at producing zero-phase lag synchrony than mutually excitatory networks

(van Vreeswijk et al., 1994). Periodic post-synaptic inhibitory potentials have been observed in hippocampus (Soltesz and Deschenes, 1993; Ylinen et al., 1995a,b) and neocortex (Steriade et al., 1993a). Oscillatory membrane potentials can be observed intracellularly in V1 cortical neurons in cat during visual stimulation, and probably represent a synchronous pattern of excitatory (and possibly inhibitory) synaptic input to the neuron (Jagadeesh et al., 1992). However, the neuronal connectivity that might be responsible for these interactions has yet to be fully demonstrated.

Intrinsically oscillatory neurons, or "pacemaker" neurons, may also be involved in the oscillatory synchronization of cortical networks. A subpopulation of bursting visual neurons in primary visual cortex, called "chattering cells", has been proposed to play a pacemaker role in the ~40 Hz synchronization of its pyramidal neighbors (Gray and McCormick, 1996). Similar properties have been observed in neurons in association cortices (Steriade, 1997; Steriade et al., 1996a, 1998), and in thalamocortical neurons of the intralaminar nuclei (Steriade et al., 1993b). Such properties in thalamocortical neurons support hypotheses of the involvement of these neurons in cortical synchronization (Llinas and Ribary, 1993; Steriade et al., 1993b, 1996a,b, 1998).

Synchronization in other sensory systems.

Neural synchronization in a number of other areas of the brain have been suggested to play a role in both sensory encoding and attentive behavior . For example, stimulus-evoked neural synchronization may be involved in encoding sound frequency in the auditory system. Experiments performed in monkey primary auditory cortex showed that a long-lasting tone evokes only a phasic increase in the mean firing rate of neurons that occurs at the tone onset. However, analysis of the temporal correlations between neural firing patterns showed a long-lasting increase in the coordination (though non-periodic) of firing between them (deCharms and Merzenich, 1996).

In somatomotor systems, synchronized activity has been suggested to underly an attentive behavioral state. Oscillations in the population activity (which imply an underlying neural synchronization) within sensory cortex and thalamus are observed during periods of alert immobility (Bouyer, 1981, 1987; Nicolelis et al., 1995). A remarkable coherence of activity can be observed at many levels in the rat somatosensory system, as shown by multiple recordings from brainstem, thalamic and cortical neurons during normal behavior (Nicolelis et al., 1995). These recordings reveal distributed spatiotemporal patterns occurring in response to tactile stimulation and prior to the onset of exploratory movements, suggesting that such activity patterns may be involved both in encoding tactile information and the coordination of the sensory input during in active sensation (Nicolelis et al., 1995).

Experiments in the motor cortex of awake behaving monkeys reveal field oscillatory activity prior to the execution of trained and voluntary movements (Murthy and Fetz, 1992; Murthy et al., 1992; Donoghue and Sanes, 1991; Gaal et al., 1992; Sanes and Donoghue, 1993). During complex exploratory tasks requiring attention (extracting hidden raisins by touch) oscillatory activity increased in amplitude, indicating an increase in the degree of underlying neural synchronization. Inter-areal synchronization was also observed between neurons in motor and somatosensory cortices (Murthy and Fetz, 1992; Murthy et al., 1992).

Multiple simultaneous single-unit recordings in the motor cortex of macaques during motor tasks have further strengthened the view that synchronization maybe be important during attentive, but motionless, phases of behavior (Riehle et al., 1997). In these experiments, coincident spiking events occurred more frequently during the delay phase, just prior to an expected event. This is suggested to be a reflection of the internal cognitive state of the animal, since it occured in the absence of any outward stimulus (e.g., a light flash, etc.). Furthermore, increases in coincident firing were correlated with the

actual behavior of the animal; its errors, and thus its failure to predict the event, were correlated with a lack of coincident firing.

Hippocampus.

Although the functional role of oscillatory activity in the hippocampus is not yet clear, the mechanisms involved in the generation of oscillations provide some guidance for our understading of neocortical oscillatory synchronization, including a possible mechanism for long-range synchrony that might provide a solution for interhemipheric synchronization (Engel et al., 1991a).

Data from the hippocampus support the hypothesis that inhibition helps shape the ~40 Hz (fast) oscillations in cortical networks (Whittington et al., 1995). Whittington et al. (1995) showed that an isolated network of inhibitory interneurons connected by $GABA_A$ synapses can oscillate at 40 Hz when driven by metabotropic glutamate application, or tetanic stimulus. Activation of networks of inhibitory interneurons with glutamate application evokes rhythmic fast IPSPs in pyramidal cells and synchronizes these neurons by entraining their firing. The effects on pyramidal neurons are thought to arise from the coherent activity of mutually inhibitory interneurons, based on analysis of the complex IPSPs evoked in widely separated cells. This idea is supported by modelling data exploring mutually interconnected inhibitory interneurons (Whittington et al., 1995; Wang and Buzsáki, 1996). Some evidence suggests these mechanisms extend to neocortical circuits as well (Whittington et al., 1995). An entrainment effect of GABAergic neurons onto pyramidal cells has been demonstrated directly (Cobb et al., 1995). A GABAergic interneuron can effectively entrain a postsynaptic pyramidal cell at theta frequencies; moreover, a single neuron could entrain two simultaneously recorded pyramidal cells, synchronizing them.

A modelling study based on these results introduced a novel mechanism for long-range synchronization: spike doublets in the inhibitory interneurons were found when the

system synchronized (Traub et al., 1996). The existence of spike doublets in these neurons was confirmed in hippocampal slices (Traub et al, 1996).

Do neurons detect correlated activity?

If neural synchronization is to be functionally useful, the neurons downstream from the synchronous ensemble should be sensitive to temporal correlations in their inputs. The results presented in this thesis work (Chapter 3) is one experimental effort at examining whether neurons can be sensitive to the level of synchronization among their inputs. Modelling methods have been employed to ask whether synchronized inputs are more effective then asynchronized inputs at driving a cortical neuron (Bernander et al., 1994; Murthy and Fetz, 1994). The answer depends on the relationship between the time constant of the membrane, the decay time of postsynatpic potentials, and rate and degree of synchronization of the inputs (König et al., 1996; Bernander et al., 1994; Murthy and Fetz, 1994). The smaller the membrane time constant and the faster the decay of the potentials, the more inclined the system is toward the detection of spike coincidences. However, as the number of coincident spikes excedes the number required for reaching threshold for an action potential, then firing rate does not continue to increase (Bernander et al., 1994). It should be noted, however, that the above results consider synaptic integration in the case of *passive* membrane properties. Active currents in the dendrites may boost the detection of coincident inputs, and such active properties have been found in the dendrites of pyramidal neurons (see review by Yuste and Tank, 1996) and in the Kenyon cells of the insect mushroom body (Laurent and Naraghi, 1994).

In summary, neural synchronization can be observed in many brain areas. Behavioral evidence suggests that it may have a functional role in sensory coding in visual, auditory, and sensorimotor systems. The degree of activity synchronization is also correlated with attentive behavioral states, implicating synchronization in selective

attentional processes or awareness. The mechanisms responsible for dynamic stimulus- and state-dependent synchronization have yet to be fully demonstrated, but likely involve network interactions such as excitatory and inhibitory reciprocal synaptic connectivity.

Chapter 2: Origin and mechanisms of synchronization of antennal lobe neurons.

Introduction.

Although stimulus-evoked oscillatory synchronization of neuronal assemblies has been observed in many sensory systems, the mechanisms underlying coherent recruitment of neurons still remain elusive. Most current models of oscillatory synchronization involve some type of inhibitory interaction, often via reciprocally connected networks of inhibitory (generally GABAergic) and excitatory neurons (Rall and Shepherd, 1968; Freeman, 1975; Lytton and Sejnowski, 1991; Bush and Sejnowski, 1996; Whittington et al., 1995; Cobb et al., 1995; Bal et al., 1995a,b). Models of networks of mutually inhibitory neurons are capable of oscillating synchronously at both low (Wang and Rinzel, 1992) and high frequencies (van Vreeswijk et al., 1994; Whittington et al., 1995; Wang and Buszaki, 1996). Such networks could then impose a widespread, synchronizing influence on their post-synaptic targets. One fundamental observation necessary for such a mechanism, namely periodic IPSPs, has been made in hippocampus (Soltesz and Deschenes, 1993; Ylinen et al., 1995a,b) and neocortex (Steriade et al., 1993b; Silva et al., 1991; Llinás et al., 1991). In addition, a model of thalamic spindle oscillations has been developed in great detail: membrane properties, synaptic kinetics, and channel conductances have been described from intracellular physiology *in vitro*, and realistic modelling has lead to an understanding of the interplay between intrinsic and network properties in producing collective behavior of the reciprocal excitatory-inhibitory circuitry (Bal et al., 1995a,b; Destexhe et al., 1996; Bal and McCormick, 1996). For all these studies, no one has conclusively shown that the synchronized oscillatory activity involved in normal neural responses can be disrupted by directly antagonizing or affecting any crucial component of

these mechanisms. Consequently, it has not yet been possible to selectively alter or suppress the synchronization of such assemblies *in vivo*, a step essential to test their functional significance for neural coding. The possible function (if any) of neural synchronization thus remains largely unknown, and will be addressed in this work.

What causes the oscillations in the locust olfactory system?

Field potentials are generally thought to be the averaged, extracellular potential due to coherent synaptic current loops (Mitzdorf, 1985). Oscillatory field potentials recorded in the MB calyx could then be caused by two different (non-exclusive) types of activity: 1) they could be generated in the MB itself and be due to intrinsic synaptic activity among Kenyon cells; or 2) they could be generated by the input to the MB, i.e., by the synaptic effects of synchronized and periodically firing PNs onto their targets, the KCs.

Data on the local circuitry in the AL, however, show that oscillatory synchrony can be seen in the AL itself. Physiological evidence will show that local interneurons (LNs) of the antennal lobe have direct inhibitory effect on PNs, and thus that they serve as prime candidates for governing synchronization in this system.

Results.

Oscillatory activity can be observed in both the PNs and LNs (Laurent and Davidowitz, 1994; Laurent et al., 1996), and immunohistochemical evidence suggested that the LNs are GABAergic (Leitch and Laurent, 1996). We investigated the nature of the effects of the local interneurons on the PNs. Intracellular labelling of local neurons revealed extensive dendritic arbors throughout the entire antennal lobe neuropil, providing a potential morphological substrate for widespread synchronization (Fig. 1A). Simultaneous intracellular recordings were made from synaptically connected local and projection

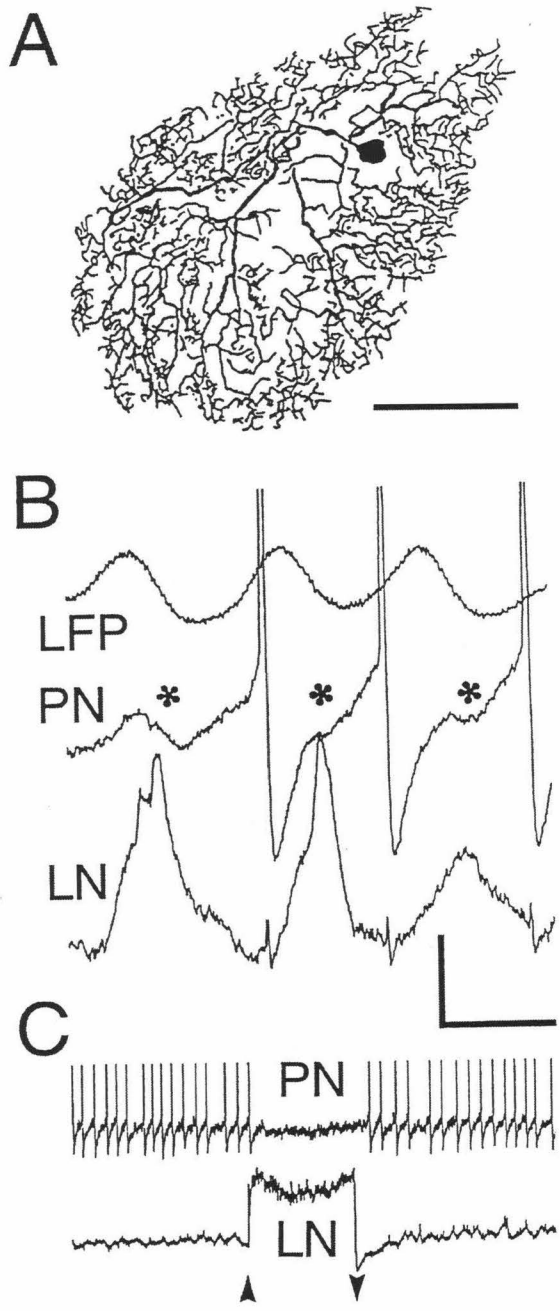

Figure 1. Local neurons inhibit projection neurons monosynaptically. (A) Camera lucida drawing of a local neuron stained intracellularly from a dendrite by injection of cobalt hexamine. Dense, extensive arborizations occupy the entire neuropil, and no axon is present. Bar, 100 mm. (B) Simultaneous intracellular recordings from an antennal lobe local neuron (LN) and a postsynaptic projection neuron (PN), as well as the local field potential (LFP) from the ipsilateral mushroom body, during a response to a cherry odor *in vivo*. PN spikes are clipped. Calibrations : horizontal, 40 ms; vertical, 200 mV (LFP), 4 mV (PN), 5 mV (LN). (C) Direct current injection pulse (between arrowheads, 600 pA) in the LN [same pair as in (B)] inhibits tonic firing of PN (held depolarized by 300 pA constant current to evoke tonic spiking). The experiment was carried out in the absence of odor. The LN does not oscillate intrinsically upon depolarization. PN spikes are clipped. Calibrations: horizontal, 0.7 s; vertical, 32 mV (PN), 18 mV (LN). This LN dye fill and these recordings were done by G.L.

neurons *in vivo* (n=4 pairs) (Fig. 1B). They revealed that, during odor responses, the timing of the periodic depolarization in LNs corresponds precisely to that of the periodic hyperpolarization in postsynaptic PNs, and showed directly that LNs lead PNs by a quarter period (96°± 53°, mean±std; n = 164 cycles), as predicted for olfactory bulb circuits (Freeman, 1964; Rall et al., 1966; Rall and Shepherd, 1968). Injecting depolarizing current directly into individual LNs evoked sustained inhibition in postsynaptic PNs (Fig. 1C). Transmitter release from LNs is spike independent and graded. Hyperpolarizing one LN never abolished the periodic inhibition of a postsynaptic PN during an odor response. Hence, the periodic inhibition of any one PN during an odor response must result from converging inhibitory input from many LNs. This can be seen in Fig. 1B also, where the size of each periodic inhibitory postsynaptic potential (IPSP) in the PN (asterisks) was not closely correlated with that of the corresponding depolarization of the impaled presynaptic LN. These results, along with immunohistochemical results (Leitch and Laurent, 1996), support a model of antennal lobe circuitry as follows: a PN receives inhibitory input from multiple LNs, and excitatory input from olfactory receptor neurons and possibly other PNs, while LNs receive input from PNs, other LNs, and possibly receptor afferents as well. The dynamics of such interconnected networks are being studied via realistic modelling studies elsewhere.

To elucidate the mechanisms underlying synchronization in the locust olfactory system, we wished to address two main questions. First, where do the oscillations observed in mushroom body local field potential recordings and in the neurons' membranes originate? More specifically, are they due only to the local feedback inhibition between antennal lobe LNs and PNs, as described above, and as hypothesized for the vertebrate olfactory system? Second, if we disrupt these putative synchronizing synaptic circuits, do we also disrupt the spatiotemporal patterning of the odor-evoked activity which characterizes PN ensembles?

To answer these questions, we studied the effects of a known antagonist of the insect ionotropic γ-aminobutyric acid receptor, picrotoxin (PCT). Other synaptic receptor antagonists were also used, and their effects will be described later. We recorded local field potentials from the mushroom body calyx to study population activity, and simultaneously, intracellularly from both types of antennal lobe neurons (PNs and LNs) before and after application of the antagonist. The following results suggest that inhibitory synaptic interactions within the AL are crucial for synchronization.

Synchronous oscillations originate in the antennal lobe.

To determine the site of origin of the odor-evoked oscillations observed in the mushroom body, we performed local injections of the GABA receptor antagonist, picrotoxin (PCT), into the antennal lobe and into the mushroom body calyx (region from which the LFP was recorded). We first recorded odor-evoked LFPs and calculated from them the power spectral density during odor-stimulation (Fig. 2A,i) for 10-30 trials per odor. A small (~1pl) volume of 0.1mM picrotoxin/saline solution was then injected into the ipsilateral antennal lobe neuropil. The LFP oscillations quickly disappeared (0.5-3 minutes), as confirmed by the spectral analysis (Fig. 2A,ii). To control for the possibility of artifactual mechanical disruption of the circuits (independent of PCT), we performed experiments where an equal volume of pure saline was injected into the antennal lobe (n = 4; Fig. 2B). This manipulation had no effect on the odor-evoked oscillations. Similarly, injections of PCT into the mushroom body calyx had little effect on the odor-evoked LFP oscillations (Fig. 2C). To check that the injections were restricted to the neuropils of interest, we performed similarly prepared and sized injections of a dye (cobalt hexamine), allowed 1-2 minutes for diffusion, and processed for histological intensification. These experiments showed that the drugs injected in this manner were restricted to the antennal lobe or calyx neuropil, probably due to the efficient barriers posed by the surrounding glial layers.

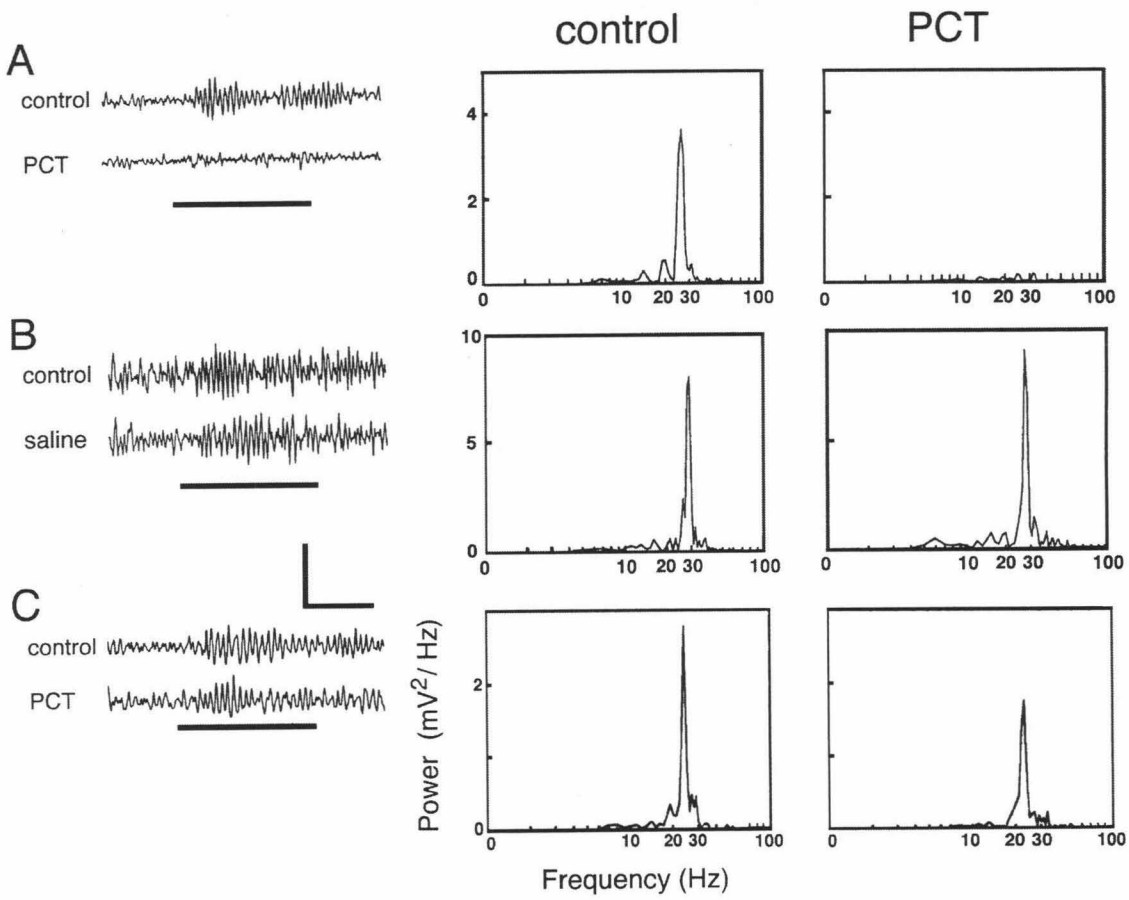

Figure 2. Injection of PCT into AL, but not mushroom body, eliminates LFP oscillations. (A-C) Left: traces of LFP recordings and during odor stimulation (horizontal bar). Right: Power spectra from individual trials at the left (note logarithmic scale on x-axis). (A) LFP recordings before (i) and after (ii) injection of PCT into the AL. Oscillations show a large peak at ~28 Hz in control (i) are absent after injection (ii). (B) LFP recordings before (i) and after (ii) injection of saline into the AL. (C) Injection of PCT into the calyx of the mushroom (next to LFP recording site) does not eliminate peak in spectrum. Different animal in each experiment. Calibrations: horizontal, 0.5 s; vertical, 1 mV (A,B), 250 μV (C).

Ablation experiments in which the MB calyx is removed do not eliminate oscillatory potentials in the PN recordings (Laurent and Davidowitz, 1994). As in the OB (Gray and Skinner, 1988), removal of potential feedback loops from higher centers suppress oscillatory synchronization of AL circuits. In addition, there is no evidence to suggest that the afferent input to the AL (from the antennal nerve) from peripheral olfactory receptors contains any fast oscillatory or synchronized components (Wehr, 1999; Wehr and Laurent, 1998). Thus, blockade of fast GABA-mediated inhibition in the antennal lobe is necessary and sufficient to eliminate LFP oscillations, indicating that the synaptic circuitry in the antennal lobe is required for their generation.

PN synchronization can be selectively eliminated by blocking fast inhibition in the antennal lobe.

In control conditions, odor puffs evoke synchronized and oscillatory activity in odor-specific sets of antennal lobe PNs, which are responsible for the burst of LFP oscillations recorded from the mushroom body. The phase-locking of an individual PN with the ensemble can be shown by its phase-locking to the LFP (Fig. 3A). PCT injection into the AL (methods as in previous section) eliminates this synchronization (Fig. 3B). Does PCT injection only eliminate PN synchronization, or does it affect other spatiotemporal aspect of the ensemble response to odors?

A trivial explanation for the elimination of LFP oscillations after PCT injection might be that the PNs no longer fire. Simultaneous recordings of the LFP and PN intracellular voltage showed that this is not the case (Fig. 3B). The transient synchronization between a PN and the LFP oscillations can be seen in a sliding cross-correlation, which represents the progressive change of the cross-correlation function during the odor response (Fig. 3A). The vertical stripes in the cross-correlation show that this PN was phase-locked to the LFP oscillations during an epoch of the ensemble response. The spacing between the stripes indicates the periodicity of the oscillation; their

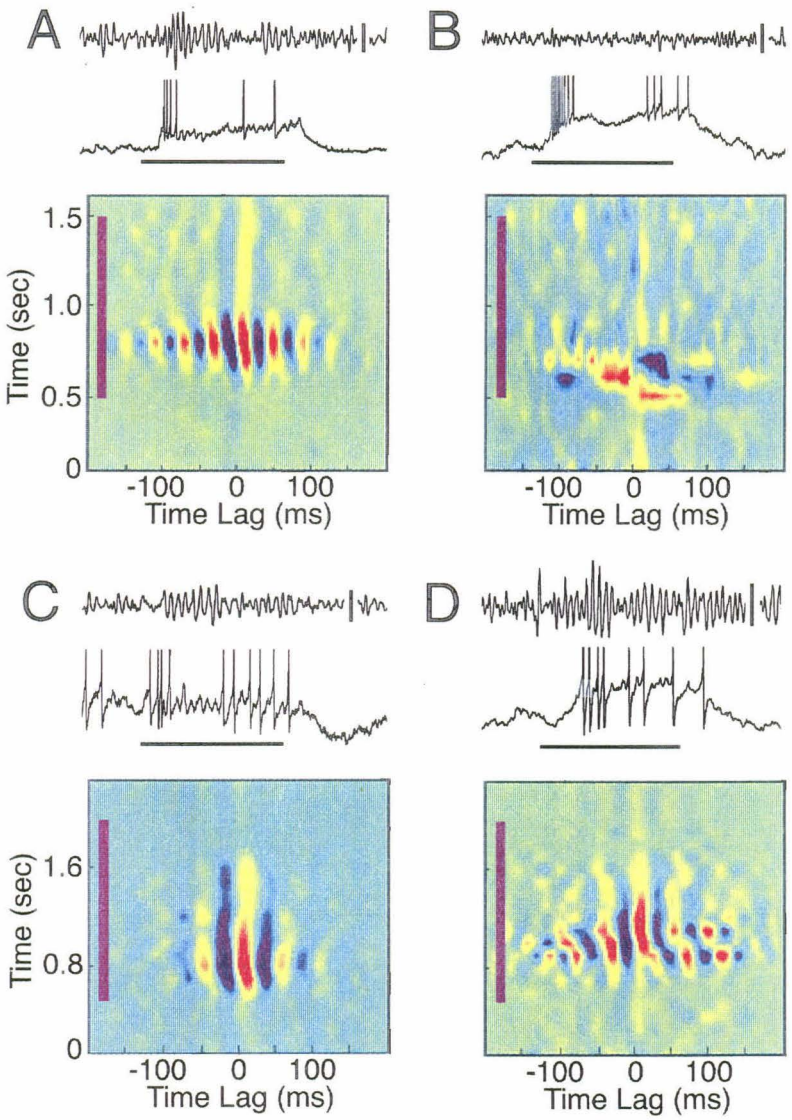

Figure 3. Picrotoxin injection in the antennal lobe selectively abolishes the oscillatory synchronization but not the responsiveness of PNs. [(A) to (D)] (top trace) LFP from mushroom body; (middle) simultaneous intracellular recording from antennal lobe PN during odor puff (indicated by horizontal bar); (bottom) sliding cross-correlation between LFP and PN traces. High values in hot colors, low values in cold colors. (A) Control response to mint odor. The biphasic PN response and the prominent IPSPs are apparent during the odor response. The oscillatory LFP indicates synchronized and rhythmic firing of many other PNs during the odor response. The cross-correlation between PN and LFP shows a striped pattern with ~50-ms period during the first half of the odor puff (vertical bar between 0.5 and 1.5 s), indicating an odor-evoked transient synchronization between this PN and the LFP. (B) Same pair as in A, after PCT. Although the response pattern of the PN to mint is not qualitatively altered, the IPSPs have disappeared, and the 20-Hz LFP oscillations are abolished. The cross-correlation is aperiodic, indicating desynchronization of the PN ensemble representing mint. (C) Local injection of saline into the antennal lobe has no effect on synchronization and LFP oscillations. Different animal from that in A and B. (D) Local injection of PCT into the calyx of the mushroom body does not affect either the responsiveness of PNs to odors or their synchronization. PN spikes are clipped. Calibrations (electrophysiological traces): horizontal, 1 s; vertical (in mV): LFP, 0.2 [(A) and (B)] , 0.5 (C), 0.3 (D); PN, 10 [(A) and (B)], 5 (C), 30 (D)

position relative to zero-time-lag indicates the phase of one signal relative to the other. After PCT application, it was observed that the odor-evoked activity is not eliminated (Fig. 3A,B lower trace; Figs. 4, 5, 6), while the oscillations in the LFP are eliminated (Fig. 3A,B upper trace). The phase-locking of this PN to the LFP is consequently disrupted (Fig. 3B, cross-correlation). In fact, PCT application, whether injected into the AL (n = 6) or bath-applied (n = 20), never eliminated PN responses. Saline injections had no effect on either the LFP (Fig. 2B, Fig. 3C), the PN response (Fig. 3C), or the phase-locking between them (Fig. 3C). Injection of PCT into the calyx of the mushroom body (n = 6) also did not block the odor-induced oscillatory responses of PNs (Fig. 3D), indicating that PN synchronization does not depend on fast GABA-mediate inhibitory feedback in the mushroom body. The suppression of odor-evoked LFP oscillations by PCT, therefore, resulted not from a silencing of the PNs, but most likely from their desynchronization, caused by the block of GABA-mediated inhibition.

Distinct mechanisms for oscillatory synchronization and inhibitory sculpting of PN activity.

PNs generally respond to odors with complex temporal firing patterns that often include discrete periods of silence. We assessed whether these slower temporal patterns, apparently sculpted by inhibition, also depend on PCT-sensitive inhibition in the antennal lobe. Three examples of PNs with such responses and of the effects of PCT on these responses are shown in Fig. 4. Although local AL injection or bath application of PCT at millimolar concentrations abolished the LN-mediated periodic IPSPs (Fig. 3B; Fig. 4, arrowheads) and the synchronized firing of the PNs (resulting in the suppression of LFP oscillations), PCT had no significant qualitative effect on the slow temporal response patterns of the PNs (Figs. 3B, 4, 5, 6). Even though the LN-mediated IPSPs responsible for PN synchronization disappeared in PCT, the timing and duration of the PN responses remained unchanged and odor-specific, as seen both in intracellular responses (Figs. 4, 5)

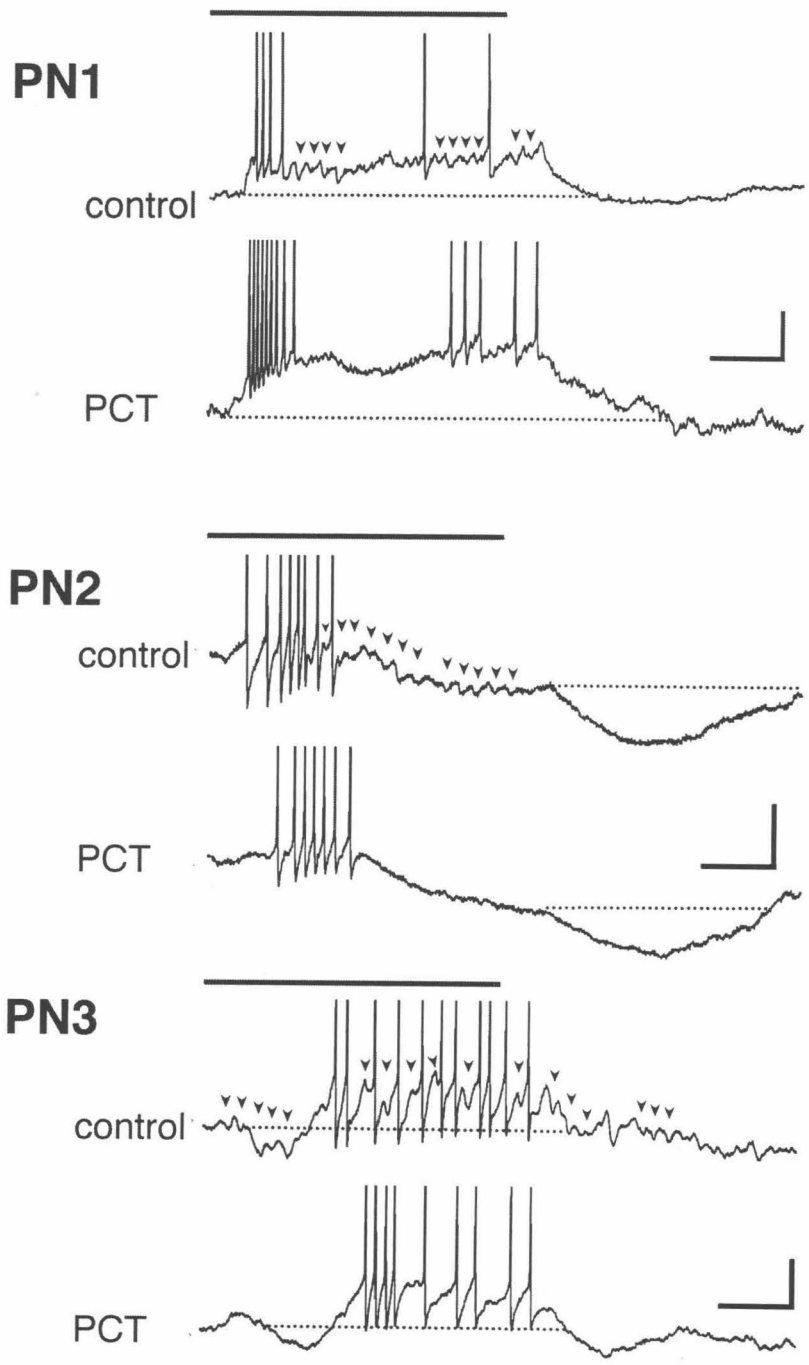

Figure 4. IPSPs evident in control responses are absent (or greatly diminished and non-rhythmic) in PCT. Calibrations: horizontal, 250 ms; vertical, 2 mV.

and peristimulus time histograms (Fig. 5) of spike activity constructed from repeated presentation of an odor (n = 26 PNs).

The slow odor-specific depolarizations and hyperpolarization of PNs looked remarkably similar before and after PCT. For example, the PN1 in Fig. 4 (same neuron shown in Fig. 3A, B expanded) responded to the odor in the control trials with a burst of spikes, followed by a several hundred millisecond-long silence, and another burst of a few more spikes late in the stimulus. The IPSPs (arrowheads) are particularly evident in the period between bursts. In PCT, the IPSPs were either eliminated or greatly diminished, but the pattern of firing remained unchanged. Note that the intervening period of silence persists, even though an underlying depolarization is maintained (Fig. 6A). In other words, the fast IPSPs blocked by PCT are not themselves responsible for the suppression. Two other examples are shown in Fig. 4 (PN2 and PN3). The response of PN2 to a cineole odor was characterized by a transient burst of spikes which ended about 500 milliseconds into the 1 second-long stimulus. Because we are recording intracellularly, we can also note the IPSPs during the second half of the stimulus, and the deep, long hyperpolarizing potential at the offset. In PCT, only the fast IPSPs were eliminated from the response. The early burst persisted, and ended at a similar average time, suggesting that the fast, PCT-sensitive IPSPs did not function to end the burst, or silence the PN. The deep, smooth hyperpolarizing potential at the stimulus offset also persisted, suggesting the existence of at least one PCT-insensitive inhibitory mechanism. This mechanism is likely to be synaptic and not intrinsic because this slow inhibition can be seen in the absence of preceding spike activity (e.g., Fig. 4, PN3). For example, in some cases, while the fast, PCT-sensitive IPSPs can clearly regulate the firing of the PN (e.g., maintain a regular firing rate), the timing of bursts (e.g., delayed onset) is regulated by PCT-insensitive potentials (e.g., Fig. 4, PN3). Eliminating the fast IPSPs with PCT almost never (53/54 odor-neuron pairs) altered the PN's firing pattern by advancing or prolonging the bursts already present. In one simple case, PCT suppressed a short interruption of a burst.

The PN firing patterns were consistent over many trials and persisted in PCT (Fig. 5). Four different PNs are shown (each one different from data shown so far), each with a different firing pattern to different odors. These patterns are representative of the range of response patterns, usually consisting of multiple periods of excitation and/or suppression. The histograms below each pair of traces represent the peri-stimulus time histograms of mean firing rate (+std), in bins of 100 ms. While bin by bin differences might be noted in some cases, overwhelmingly, the patterns persisted remarkably well, as will be quantified later.

It had been shown previously that one odor can elicit different response patterns in different neurons in the same animal (i.e., patterns are neuron-specific) and that single PNs can respond to different odors with a distinct pattern for each odor (i.e., patterns are odor-specific) (Laurent et al., 1996). PCT does not disrupt this odor- and neuron-specificity. In the example in Fig. 6, cineole, cherry, and isoamyl acetate each elicit reliable, but distinct, patterns. Similar to the examples in Fig. 5, the differences between control and PCT patterns elicited by the same odor appear negligible when compared to those evoked by different odors. Odor-specificity, qualitatively defined, is maintained despite a lack of fast IPSPs. These results will be quantified and further discussed in the next chapter.

We stated above that PCT never eliminated odor responses. But are novel responses to odors induced by PCT? We will call a change in a neuron's "tuning" or "response spectra" a change in the set of odors to which it does or does not respond (as defined by supra- or sub-threshold activity). In this sense, it has been shown previously that PNs are tuned: they respond to some and not at all to other odors with which they are tested (see Table 1 in Laurent and Davidowitz, 1994). We tested here whether a PN's tuning changes in PCT by recording both responses and "non-responses" first during control and then later during PCT trials (n=10). We found no changes in their tuning (i.e., no response induction), which suggests that the set of PNs which respond to any given odor in control conditions is identical to that which responds after PCT application.

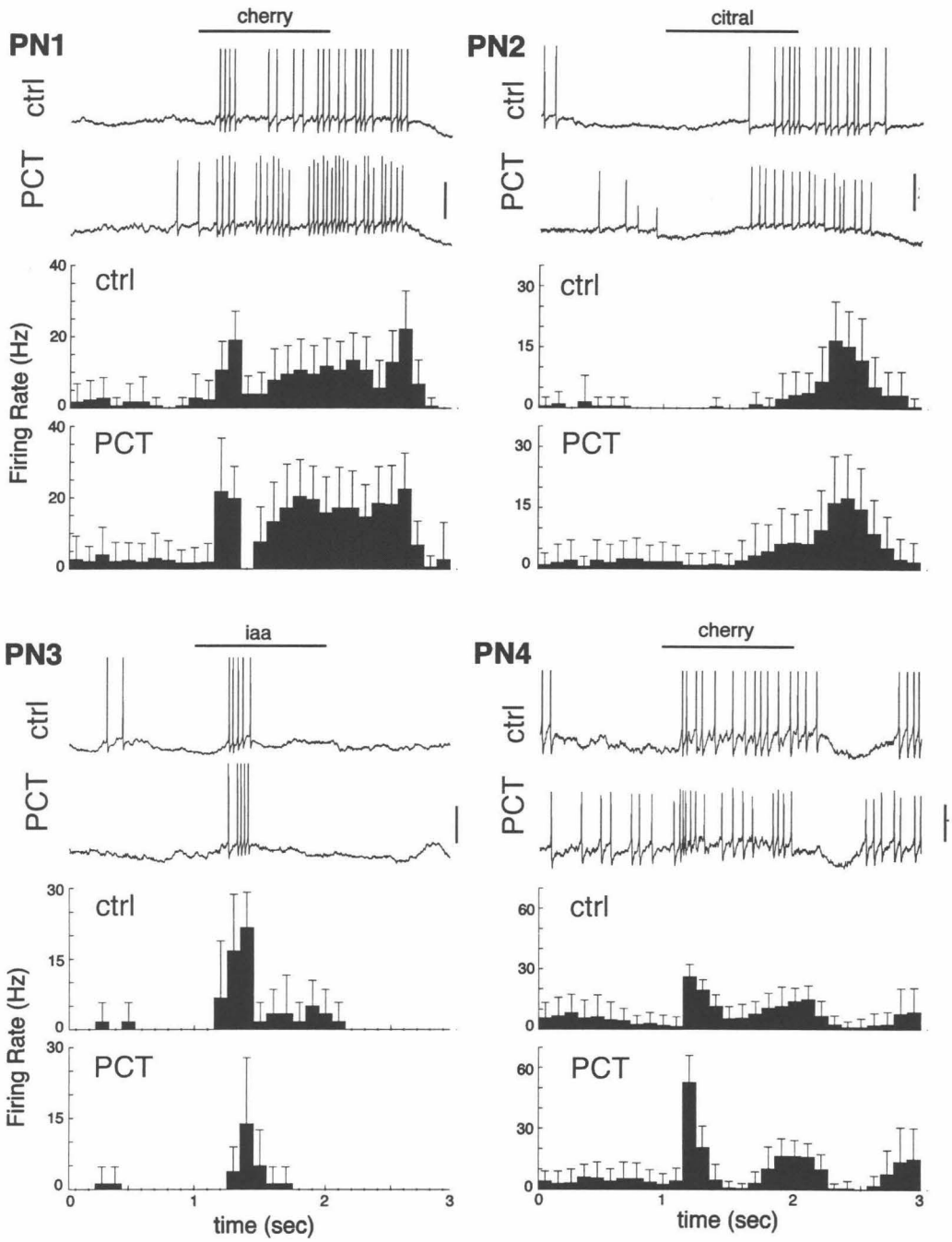

Figure 5. PN responses have slow temporal patterns in control which persist in PCT. Four PNs, each one from a different experiment. Odor stimulus indicated by horizontal bar. Mean+std peri-stimulus histograms. Calibrations (electrophysiological traces): horizontal, 1 s; vertical, 20 mV (PN1-PN3), 10 mV (PN4). PN spikes are clipped.

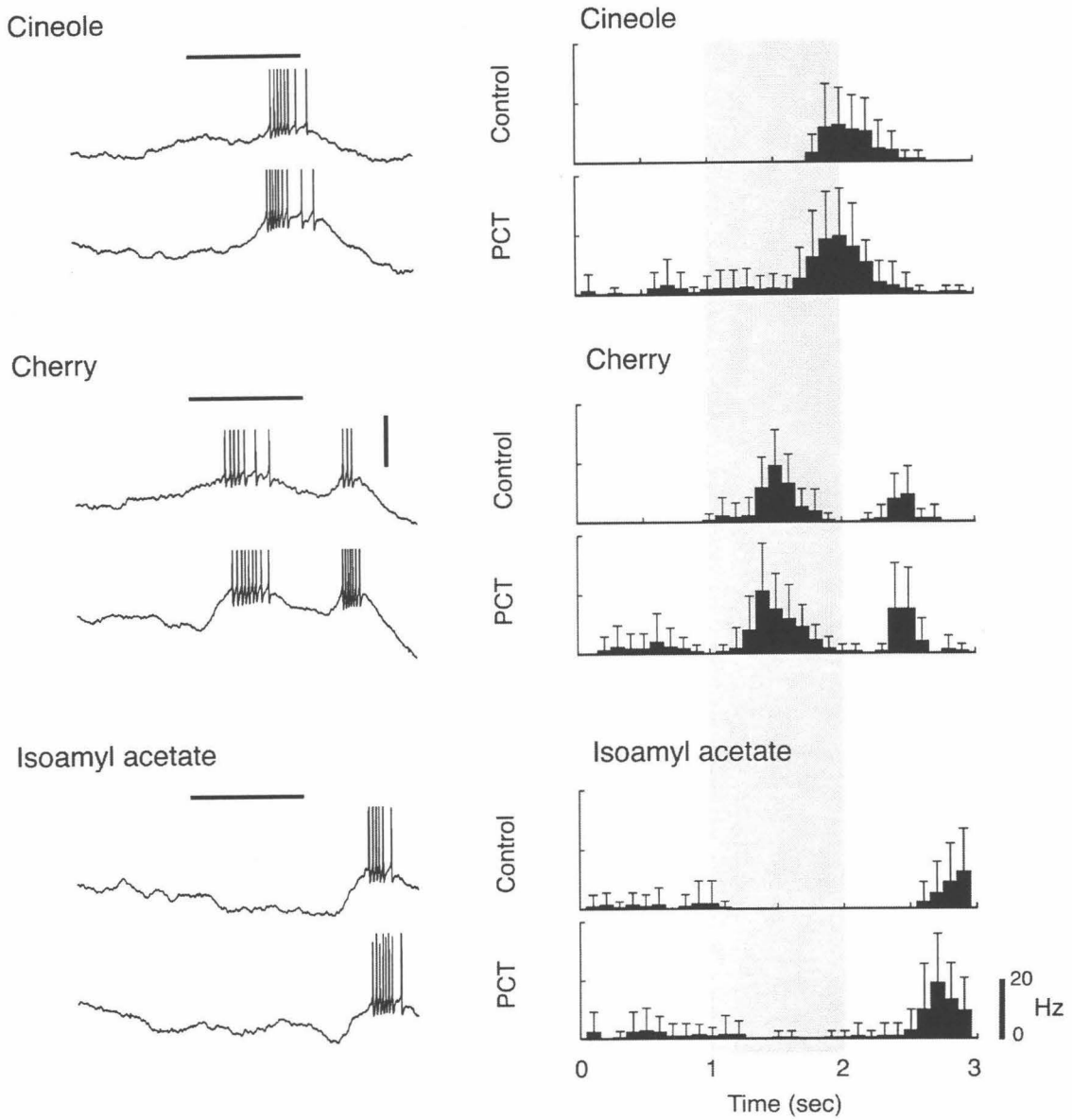

Figure 6. PN odor tuning in slow temporal patterns persists in PCT. Responses of a single PN to three different odors, before (control) and after (PCT) picrotoxin application. PST histograms represent mean spike rate+std. Shaded region indicates odor presentation. Calibrations: horizontal, 1 s; vertical, 40 mV.

The fast, PCT-sensitive IPSPs in the responses of the PNs clearly seem to regulate their firing. Indeed, their function lies in delaying the action potential sufficiently and simultaneously in multiple PNs to entrain their mutual activity and thus induce synchronization, as evident in the LFP oscillations. Therefore, abolition of these IPSPs also eliminated them as a 'brake' on the firing of the PNs. This would sometimes result in higher instantaneous frequencies in an excitatory burst (e.g., see top trace in Fig. 4). We wished to determine how often, and whether this was a significant effect on PN response properties. We first tested, in each experiment, whether PCT had any statistically significant effect on the mean firing rate of the response. We chose this measure because average firing rate is the most common metric of response quantification. The vast majority of the responses (89%) showed no change in firing rate. We next assessed whether there was a trend which, while not statistically significant in individual experiments, might be evident over all experiments. We plotted the mean ±std response rate of control trials versus that of the PCT trials (Fig. 7A), and found a very good linear fit between the two sets of data. A slope less than one would indicate a general trend to decrease the firing rate; a slope greater than one would indicate a general trend of increase in the firing rate (as might be expected with a blockade of inhibitory action). Instead, we found a slope very close to one (0.94), and a highly significant fit. Note that the error bars most often encompass the diagonal, and that some points fall slightly above it while others fall slightly below. In this analysis we included only those responses which were defined responses (a significant change from baseline activity), and therefore did not bias our results with "non-responses" which simply reflect background activity. We also tested the effect of PCT on background rates (for all experiments, including response or non-response trials), and found a significant linear fit with a regression slope close of 0.86, not significantly different from one (Fig. 7B). These results indicate that any changes in the overall firing rates due to PCT application are minimal and balanced.

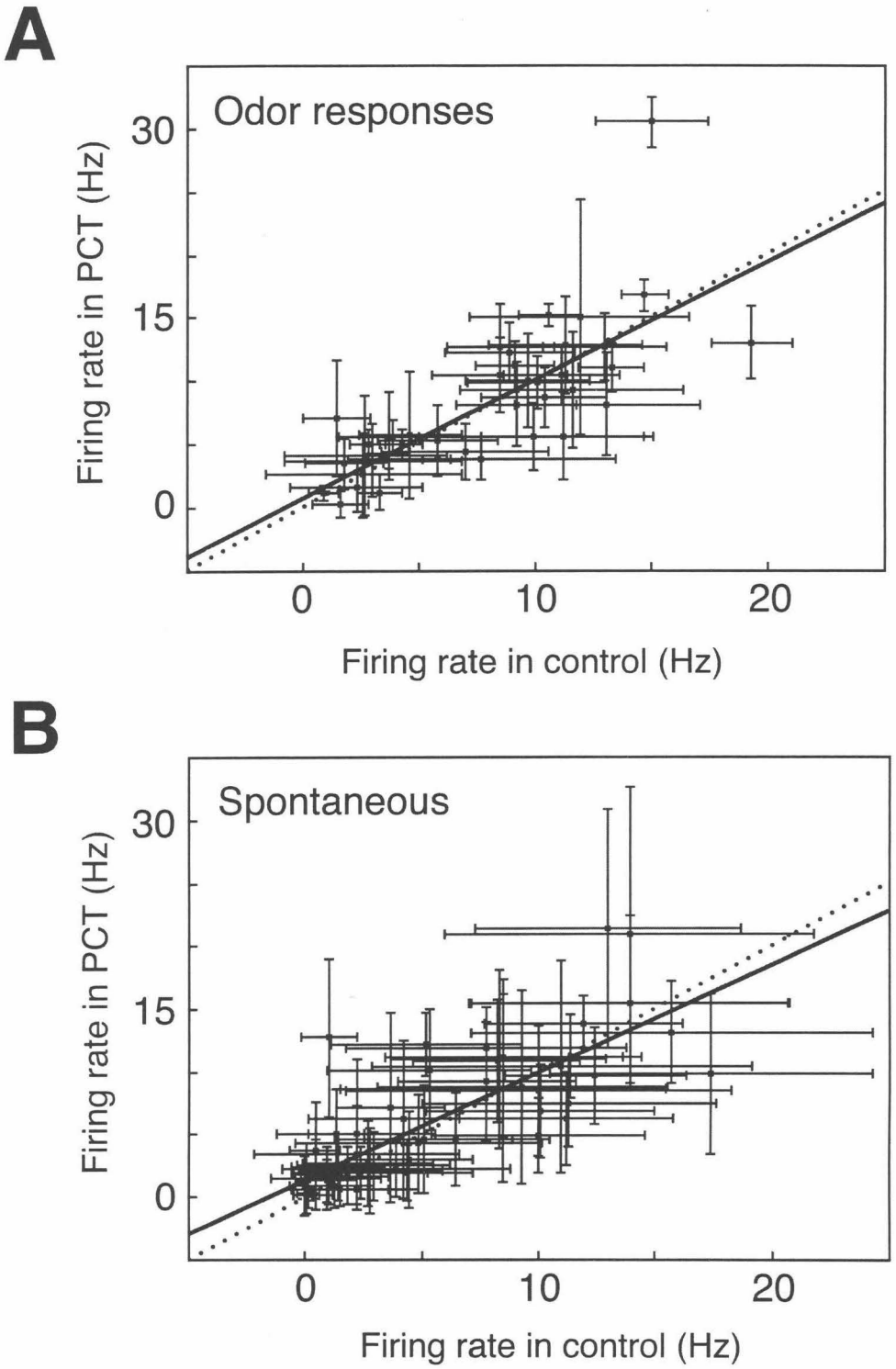

Figure 7. PCT evokes no overall change in PN odor-evoked (A) or baseline (B) mean firing rates. Each points plots firing rate in one PN before (x-axis) vs. after (y-axis) PCT injection. Regression line, solid line; diagonal, dotted line. Rates (mean ± STD) calculated over 1 sec, from 8-30 trials from 26 PNs. (A), (B): 38, 54 odor-neuron pairs respectively. (A) only includes examples where odor actually caused a response. Slope of linear regression fits: 0.86 (i, R=0.8); 0.94 (ii, R=0.76).

These results, combined with the persistence of the odor-specific patterning of PN responses, indicates that the spatio-temporal composition of odor-activated PN ensembles is maintained in PCT, except for the transient pairwise synchronization between PNs. The odor- and neuron-specific modulation of firing observed in PNs is therefore caused by mechanisms independent of PCT-sensitive GABA-mediated inhibition.

Oscillatory odor-evoked responses of the local interneurons depends on network interactions.

Odors evoke oscillatory potentials in the local interneurons (LNs) as well as in the PNs (Laurent and Davidowitz, 1994). The LNs do not spike and thus their odor-evoked responses generally consist of these oscillatory potentials, and often an underlying depolarization, which is, however, not always visible. The effect of these LNs on the PNs, to which they are connected via graded synapses, depends on their oscillatory potentials. Is PN synchronization, then, blocked because the LNs simply can no longer influence the PNs, or are LN oscillatory responses also eliminated by PCT?

There are two major mechanisms hypothesized to be responsible for synchronization in most systems. In one scheme, an intrinsically oscillatory 'pacemaker' neuron (or set of neurons) generates oscillatory membrane potentials or periodic action potentials (or bursts) and entrains the postsynaptic elements it contacts, via either excitatory or inhibitory synaptic actions (e.g., thalamic reticular and thalamocortical neurons (Steriade et al., 1993) or possibly V1 chattering cells (Gray and McCormick, 1996)). In the second scheme, generating synchrony depends on network interactions. The appropriate strength and timing of synaptic feedback interactions results in rhythmic behavior, where the exact phase relationships depend on the parameters of the system elements (the sign of synaptic interactions, the time constants of the currents, etc.) (e.g., networks of hippocampal neurons (Jefferys et al., 1996; Traub and Miles, 1991; Whittington et al., 1995)). While

the network mechanism requires no intrinsic pacemaker neuron, the two mechanisms are not mutually exclusive.

The locust AL local interneurons do not appear to be intrinsically oscillatory. Injecting a DC depolarizing current into an LN does not produce oscillatory potentials (Fig. 1C). Although depolarization-evoked oscillations have never been observed in LNs (or PNs), we cannot completely rule them out, since direct current injection may not be the appropriate way of depolarizing neurons with extensive arborizations (due to space clamp problems). On the other hand, the antennal lobe networks appear to support a large degree of synaptic interaction. The immunohistochemical data supports all possible combinations of interaction between LNs and PNs (see Chapter 1) (Leitch and Laurent, 1996). Coherent feedback from the PNs might suffice, as has been hypothesized in the vertebrate olfactory bulb (Rall and Shepherd, 1966). The immunohistochemical data shows that LNs contact one another (GABA-positive to GABA-positive synaptic profiles). It has been shown that groups of inhibitory neurons can synchronize and phase-lock with zero phase lag, as well as other phase lags, purely through inhibitory feedback (Wang and Buzsaki, 1996; van Vreeswijk et al., 1994). A subnetwork of LNs, then, might produce oscillatory activity, external to the PN population. We thus investigated the effects of PCT on the odor-evoked activity of the local interneurons.

An example of an LN odor-evoked response, and the phase-locking of the LN membrane potential with the LFP oscillations, is shown in Fig. 8A. An odor, in this case cineole, causes a depolarization of the LN on which a wave of smooth oscillatory potentials is superimposed. It is difficult to determine which potentials may be EPSP or IPSP, and there is likely a combination and alternation of both, as well as active components boosting many of the depolarizing phases. A power spectrum of the response (Fig. 8C, blue line) shows a significant peak at ~20 Hz, with most of the power ranging between 15 and 25 Hz (this spectrum is an average calculated from LN traces bandpass filtered between 5 and 55 Hz). Note also the phase lag in the LN-LFP cross-correlation (Fig. 8A), unlike the lead

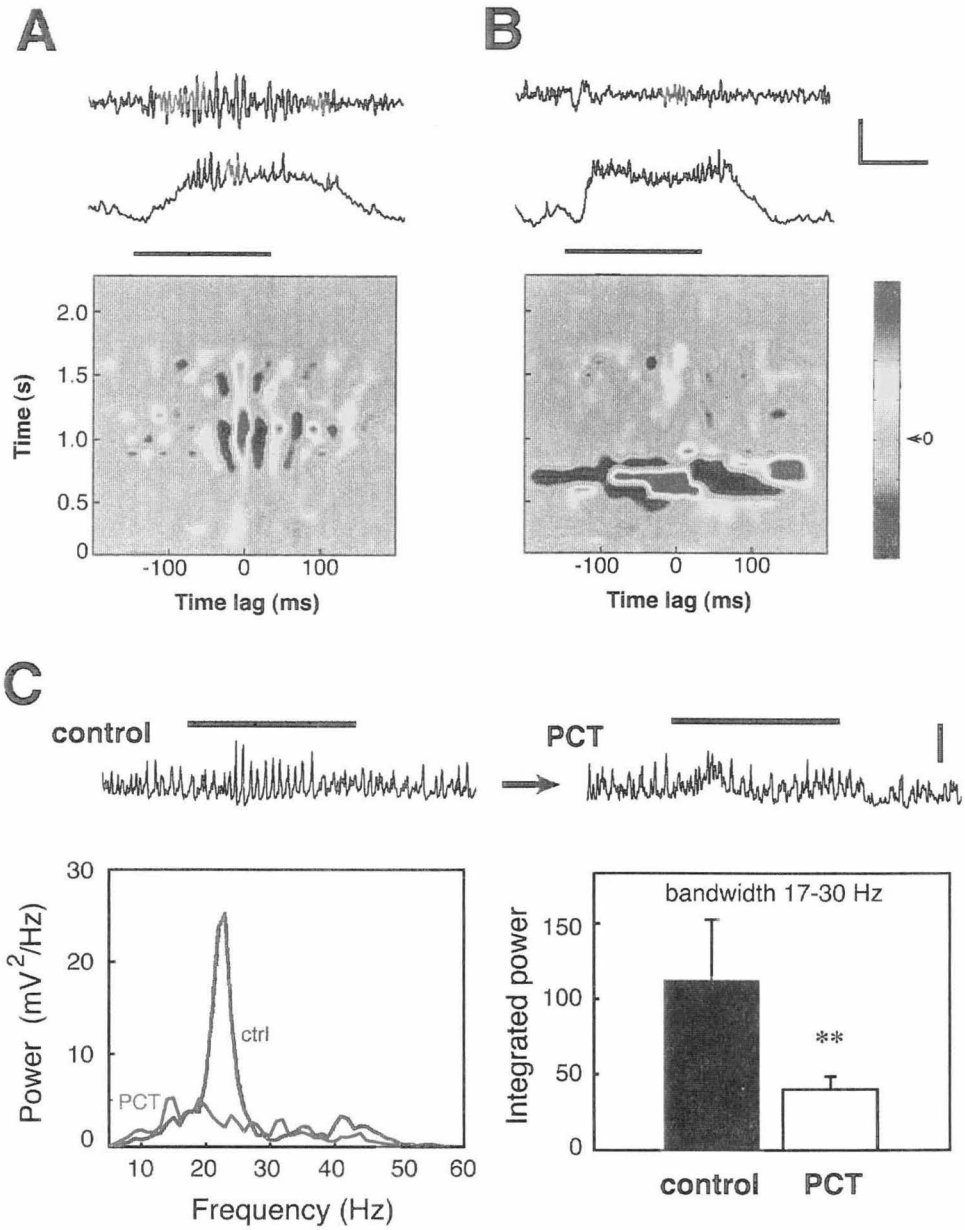

Figure 8. Local interneuron oscillatory odor response is disrupted by PCT. (A) Control LFP (top trace) and LN (bottom trace) recordings in response to a puff of mint. Bottom: The cross-correlation between LN and LFP shows a striped pattern with ~50-ms period during the odor puff, indicating an odor-evoked transient synchronization (average of 10 trials). (B) PCT injection eliminates the ~20-Hz oscillations in the LFP (top), and the ~20-Hz membrane potential oscillations, but not the slow depolarization, in the LN. The cross-correlation between them is aperiodic (average of 7 trials). Calibrations (A-B): vertical, 0.4 mV, LFP, 2.4 mV, intracellular; horizontal, 500 ms. (C) Top: Local interneurons responses in another animal to mint, before (control) and after PCT. Calibrations, vertical, 2 mV. Bottom left: Power spectra of this LN during odor responses averaged across trials for control (blue line, n=14) and PCT (red line, n=11) trials. The peak at ~23 Hz evident in control is greatly decreased in control. Bottom right: Quantification of LN power in 17-30 Hz bandwidth reveals a significant ($p<0.0001$) decrease in power in PCT (open bar) from control (black bar). Bars indicate mean±STD summed power between 17-30 Hz. (A-C) Odor stimuli (horizontal bars), all one second.

that occurs with PNs.

PCT application disrupted the 20 to 30 Hz oscillatory component of the response of this LN (Fig. 8B). This suggests that this neuron still received input due to olfactory stimulation, but shows that response spectrum is modified. PCT eliminated neither the slow depolarizing potential nor some of the "spikelet" activity superimposed on the depolarizing plateau. This remaining activity was not phase-locked to the LFP (Fig. 8B). An average power spectrum of the PCT trials (Fig. 8C, left, red line) shows an elimination of the peak, or a decrease in power, at ~20 Hz. In 3 of 4 LNs (and for 5 of 8 odor-neuron pairs) there was a significant decrease in the summed power between 17 and 30 Hz in the responses from control to PCT trails (shown for this neuron in Fig. 8C, right).
This result also supports the conclusion that the LNs are not intrinsically oscillatory (since they may be depolarized without oscillating), and that they depend on (PCT blockable) synaptic input to generate oscillatory potentials. This may result from the lack of coherence of the inputs from PNs (no longer synchronized), or possibly from the disruption of a purely inhibitory feedback network. Unfortunately, we cannot determine from the present data which of these two influences is critical (both in combination are quite likely), since a blockade of PN excitatory synaptic interactions (with an nAch receptor antagonist, for example) would probably also block input from the olfactory receptor neurons. In conclusion, it appears that network dynamics are required for the oscillatory behavior of the LNs as well as PNs, and for the PN/LN synchronization that emerges during odor stimulation.

Relationship between antennal lobe and mushroom body oscillatory properties.

The intrinsic neurons of the mushroom body, the Kenyon cells (KCs), receive on their dendrites in the calycal region of neuropil *en passant* synaptic inputs from the PNs (Leitch and Laurent, 1996). In response to odor stimulation, KCs, like the antennal lobe neurons, respond with subthreshold oscillatory potentials (Laurent and Naraghi, 1994).

Kenyon cells, however, rarely produce excitatory, spiking responses. Rather, their firing is usually suppressed during the oscillatory potentials by a tonic or periodic inhibitory input. This is especially clear when a Kenyon cell is held to a depolarized potential with current injection, and an odor presented is during the induced tonic firing (Laurent and Naraghi, 1994). If the KC does spike, an action potential usually occurs no more often than once per two cycles of the oscillation. The oscillatory membrane potentials and the spikes they underlie are phase-locked to the MB LFP (Laurent and Naraghi, 1994).

KCs do not appear to express intrinsic oscillatory properties, as revealed by intracellular current injection (Laurent and Naraghi, 1994). Suppression of firing during odor stimulation indicates an inhibitory component to the response, and the responses of KCs to an odor often comprise an early and maintained inhibitory component. Because the PNs and are excitatory, inhibition of the KCs is unlikely to be due to direct PN synaptic action. Immunocytochemical examination of synapses in the dendritic region of the mushroom body indicated that the KCs receive direct GABA-containing inputs from neurons other than the PNs (Leitch and Laurent, 1996). Odor-evoked KC inhibition may result then, at least in part, from a feedforward circuit.

Inhibition in the mushroom body might also contribute to synchronization of the KCs receiving coherent inputs from PNs. To examine this possibility, we superfused the brain with PCT, thus blocking inhibition both in the antennal lobe and the mushroom body and monitored PN synchronization using the MB LFP (n=17). One to two minutes after PCT application, odor-evoked oscillations disappeared in the LFP because of the desynchronization of PNs in the antennal lobe (Fig. 9, A and B). A few minutes later (approximately 5-10 minutes), however, odor puffs evoked rhythmic bursts of large negative population spikes, indicative of massive synchronized firing of KCs (Fig. 9C). Power spectra of these oscillations showed that they occurred at the same frequency as control LFPs. This result indicates that the mushroom body can naturally oscillate coherently at 20 to 30 Hz in response to a desynchronized input, and that inhibition may

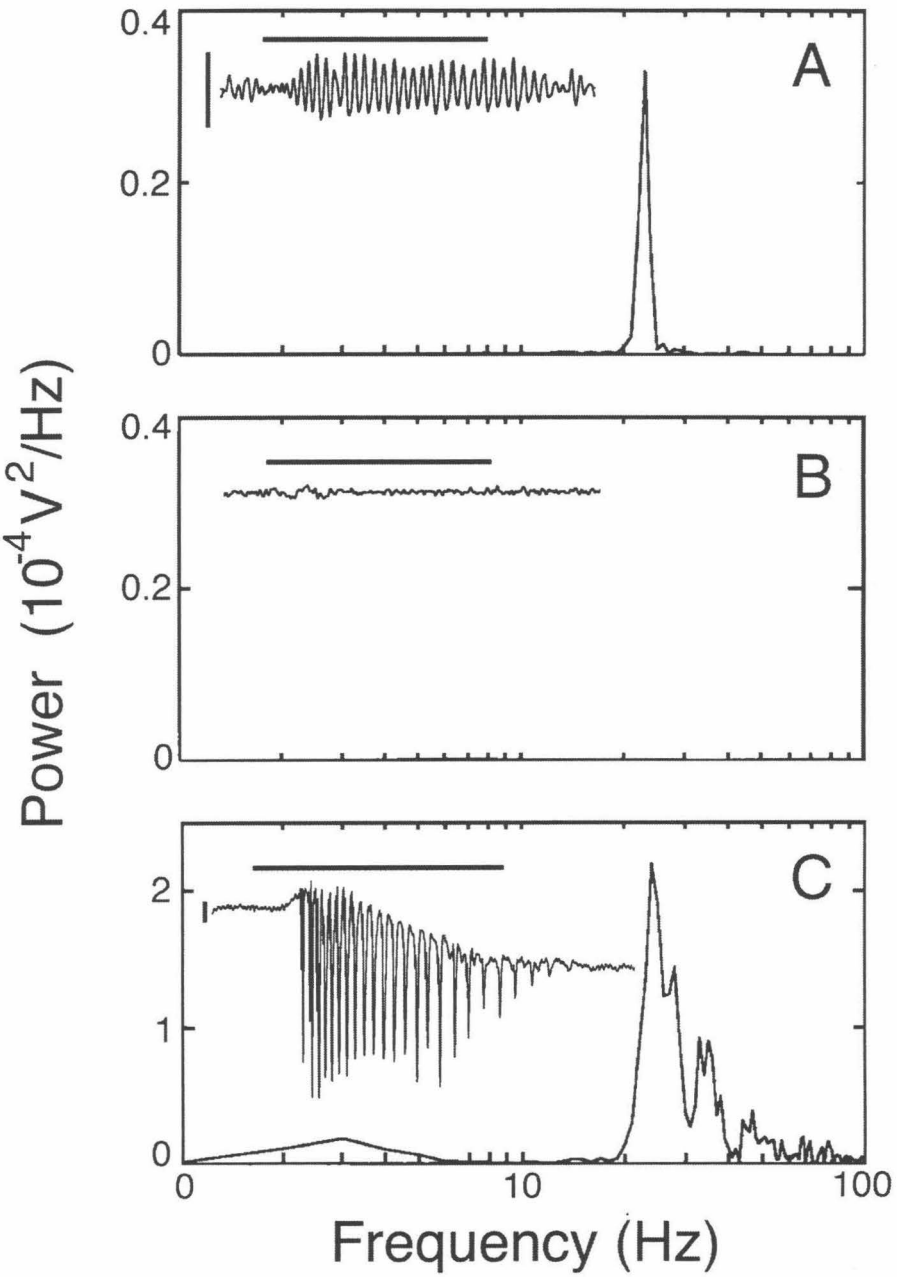

Figure 9. Effect of PCT applied simultaneously to the antennal lobe and mushroom body on synchronization and LFP oscillations. (A) Power spectrum calculated from the LFP oscillation (example in inset) evoked by a cherry odor (horizontal bar) in an intact animal (n = 20 odor presentations). A narrow peak is seen at ~24 Hz. (B) Superfusion of 1 mM PCT onto the brain of the same animal rapidly eliminates the LFP oscillations, as a result of the desynchronization of PNs in the antennal lobe. (C) Seven minutes later, odor puffs now evoke bursts of large-amplitude population spikes in the mushroom body (inset), whose power spectrum also shows a peak at 24 Hz, despite the desynchronization of PNs. Calibrations (insets): horizontal, 1 s; vertical, 500 mV.

normally control the extent of this local synchronization. Examination of the KC axon tracts revealed reciprocal synapses that do not contain GABA and are therefore most likely excitatory (Schürmann, 1974; Leitch and Laurent, 1996). Such recurrent excitation between neighboring KCs might participate in synchronizing their activity. In conclusion, it appears that MB networks may indeed have an intrinsic tendency to oscillate at a frequency similar to that of their natural AL input.

Other antagonist effects.

The vertebrate GABA receptors can be divided into two major types: one which provides a fast, Cl^--mediated conductance ($GABA_A$), and one which provides a slow, second-messenger-dependent conductance ($GABA_B$)(Hille, 1992; Sattelle et al., 1991). These receptors may also be distinguished by their pharmacology. The $GABA_A$ receptor is antagonized by bicuculline and picrotoxin, while the $GABA_B$ receptor is antagonized instead by phaclofen and saclofen (Hille, 1992; DeFeudis, 1977; Enna, 1983; Bowery, 1993). Molecular biological investigation of the structure of the vertebrate $GABA_A$ receptor has shown that it belongs to a gene superfamily of ligand-gated ion channels. In insects, the GABA-receptors are less understood. Picrotoxin, a plant alkaloid, is an effective antagonist of the insect $GABA_A$-like (Cl^--mediated) receptor, while bicuculline is often ineffective (Sattelle et al., 1991).

As shown above, in the locust olfactory system, picrotoxin blocks fast IPSPs that are caused by GABA-containing LNs. Consistent with other studies in insects which showed bicuculline insenstivity of the $GABA_A$-like rececptor (Sattelle et al., 1991), bicuculline injected into the AL (up to 7.5 mM concentrations) had no effect on the LFP oscillations.

There also exists a histamine-gated, Cl^- channel first identified in the lobster and locust nervous system (Claiborne and Selverston, 1984; Hardie, 1989; McClintock and Ache, 1989). To test whether our results may have been the result of binding of such a

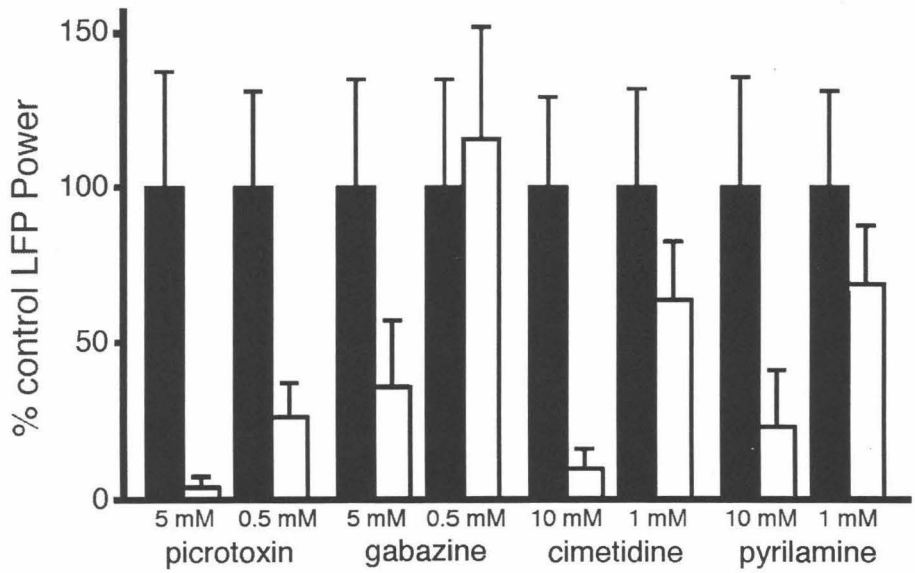

Figure 10. Comparison of effect of GABA and histamine antagonists on odor-evoked LFP oscillations. Quantification as in Fig. 8. Black bars, control trials; open bars, antagonist trials. All antagonists dissolved in saline at the concentrations indicated and bath applied. Error bars, std. Each experiment normalized to control values then averaged over three experiments for each antagonist/concentration.

histamine receptor, we tested the effect of two histamine antagonists on the LFP oscillations: pyrilamine, a blocker of the vertebrate H1 receptor, and cimetidine, a blocker of the vertebrate H2 receptor (Arrang et al., 1995). These drugs were bath-applied at two different concentrations (10 mM and 1 mM) in locust saline. For comparison we tested two concentrations of PCT, and two concentrations of a third GABA-A receptor antagonist, gabazine.

We recorded an LFP and measured odor-evoked oscillations for 13-25 trials, before and after drug application. We measured the power spectra during the odor periods of each trial, and calculated the summed power in the 20 to 35 Hz band. The results (Fig. 10) show that histamine receptor antagonists are only ~50% (pyrilamine) to ~60% (cimetidine) as effective as PCT. Gabazine is ~60% as effective as PCT at higher concentration (5 mM) but actually appeared to sometimes enhance the oscillation amplitude at low concentrations. Thus, at concentrations at which PCT reduced LFP oscillations by 95%, the effectiveness of the four drugs could be ranked as: PCT >> gabazine > cimetidine > pyrilamine. Because the insect histamine receptor channel is, like the insect ionotropic GABA receptor, a ligand-gated chloride channel, the effect of histamine receptor blockers might have been due to nonspecific binding to the GABA receptor channels.

Discussion.

These results provide experimental support to hypotheses involving inhibitory interactions in the generation of synchronized oscillations. The specific synchronizing role played by this type of inhibitory synaptic action suggests that synchronization is functionally relevant.

Possible origins of the slow temporal patterns.

While it is clear that distinct mechanisms underlie PN synchronization and slow temporal patterns, the mechanisms which control the latter remains unknown. Several mechanisms may be involved: 1) patterning in the ORN responses, 2) patterning in the odorant stimulus, 3) PCT-insensitive inhibitory pathways in the antennal lobe, 4) emergent circuit patterns.

There is no evidence from antennal nerve recordings that slow patterns exist in the ORN afferents in locust (Wehr and Laurent, 1998; Wehr, 1999). Recordings from sensory placodes (e.g., honeybee [Akers and Getz, 1992, 1993]) and electroantennograms (EAGs) in other insects fail to show such patterns, but detailed analysis is scarce. Individual variations in the patterning of responses of individual ORNs (such as latency to onset, phasic or tonic excitation, adaptation) coupled with their odor-specificity may yet explain some of the patterning at the level of the antennal lobe in response to natural odor stimuli. However, afferent patterning is not *necessary* for patterning of AL neuron responses. Electrical stimulation of the nerve can elicit patterned responses as well, suggesting that the antennal lobe circuitry itself is capable of generating them (Wehr and Laurent, 1998; Wehr, 1999). The afferent input to the antennal lobe appears to be largely unpatterned and excitatory (cholinergic) in nature. In fact, odor-specific PN responses can outlast the excitation of afferents. This suggests that AL circuit dynamics can sculpt a non-oscillatory, unpatterned input into complex, oscillatory and long-lasting patterns.

The lack of patterning in the afferents suggests a lack of patterning of the stimulus in our odor-delivery system as well. Furthermore, different PNs in the same preparation show very different patterns to the same odor stimulus, suggesting that the patterns do not arise from the stimulus itself. What would the effect of such patterning be? Some investigators have considered the behavioral and physiological effects of the temporal patterning of a pheromonal stimulus by comparing pulsed stimuli and constant stream stimuli. A behavioral study suggests that a pulsed odor presentation (which might be

similar to natural stimuli) elicits a different behavioral response in a moth than a constant odorant stream (similar to most experimental odor presentation paradigms) (Mafra-Neto and Cardé, 1994). Physiologically, antennal lobe neurons are capable of following such pulsed stimuli up to a frequency of ~10 Hz (Christensen and Hildebrand, 1988, 1997). While these results are interesting and relevant from a neuroethological standpoint, our results clearly indicate that an unpatterned stimulus elicits a patterned physiological response at the level of the antennal lobe (Wehr and Laurent, 1998; Wehr, 1999). The relationship between the temporal features of natural stimuli and the temporal patterns generated by the nervous system itself will be a very important area of research if we are to understand how the olfactory system encodes stimuli in a complex sensory environment.

Other mechanisms of inhibitory neurotransmission may be responsible for the characteristic slow and deep inhibitory components frequently seen both during and upon termination of the odor stimulation. Such inhibitory potentials did not depend on preceding excitation of the PN, and thus are not due to either intrinsic mechanisms (such as a slowly activating hyperpolarizing conductance or a pump current) or direct reciprocal feedback from an LN onto which the recorded PN synapses. Possible mechanisms might involve, for example, GABA-B type receptors, histamine receptors, or other neuromodulatory effects.

Comparison with inhibitory interactions in the olfactory bulb.

Inhibitory interactions in the olfactory bulb have been largely studied between granule and mitral cells. Granule cells have been hypothesized to play a role in both the oscillatory activity prevalent in the bulb and in lateral interactions which shape the responses of mitral cells (Rall and Shepherd, 1968). Although slow-responses patterns can also be observed in vertebrate mitral cells *in vivo* (Chaput and Holley, 1980; Meredith, 1986; Cinelli et al., 1995; Yokoi et al., 1995), it is not known whether they depend, as observed here, on synaptic mechanisms distinct from those causing synchronization.

GABA antagonists injected into the granule cell layer in the bulb induce a change in mitral cell responses by blocking their inhibitory "flanks" (Yokoi et al., 1995). Granule cells might thus be involved in the 'slow' patterning of mitral cell responses. We do not know, however, whether this manipulation had any effect on the synchronization of the population of mitral cells. A second group of putative inhibitory interneurons, the periglomerular cells, might provide a second, distinct pathway through which synchronization might be effected.

Comparison with inhibitory interactions in other insect antennal lobes.

Local interneurons have been described in several other insect species as inhibitory and GABAergic (Harrow and Hildebrand, 1982; Christensen et al., 1985; Hoskins et al., 1986; Christensen, 1993). In moth, fast IPSPs following electrical stimulation of the antennal nerve have been characterized as being mediated by GABA, i.e., chloride channel mediated and blocked by picrotoxin (and in one case, bicuculline). A recent study (Christensen et al., 1998) describes the effects of bicuculline on pheromone-evoked responses in moth PNs. They found that, while moth PNs showed dynamic responses similar to those in locust PNs, bicuculline disrupted the inhibitory component of the 'slow' patterning, resulting in tonic firing throughout the stimulus. As shown in the work presented here, in locust almost all PN odor responses showed identical stimulus response patterns after application of picrotoxin. Only *once* out of 54 odor responses studied in locust PNs did picrotoxin induce tonic in a PN whose response contained a period of inhibition There are, however, differences between the responses of moth and locust that should be pointed out. First, the receptors mediating the IPSPs in the two systems may be different: in moths, both picrotoxin and bicuculline antagonized the inhibitory potential, while in locusts, bicuculline appears to be ineffective. Second, the 'patterned' responses to prolonged (>500ms) odor stimulation in moth do not seem to vary with the identity of the odor present. They are odor-specific, in that the PN responds only to certain (pheromonal)

odorant stimuli, but the qualitative pattern may be much more stereotyped in the moth PNs. They are characterized as 'triphasic': there is an early IPSP, an EPSP, and a long delayed IPSP (itself insensitive to bicuculline and probably not Cl^- mediated)(Christensen et al., 1998a, 1998b). In locusts, however, different odors can evoke distinct, but reproducible, response patterns in individual PNs (Laurent and Davidowitz, 1994; Laurent et al., 1996; MacLeod and Laurent, 1996).

Frequency matching between the antennal lobe and the mushroom body.

We also showed that the mushroom body is independently tuned to oscillate at 20 to 30 Hz. This frequency matching between the AL and MB could enable the MB to "accept" the 20- to 30 Hz input from the antennal lobe. PCT-sensitive inhibition in the mushroom body appears to prevent stimulus-evoked runaway excitation that might otherwise result in seizures and, probably, loss of input specificity. The existence of distinct but compatible oscillatory mechanisms in the antennal lobe and mushroom body suggest that they are cooperative means to optimize synchronization and information transfer. Electrical stimulation in the lateral olfactory tract in rat piriform cortex slices evokes damped field-potential oscillations at a frequency similar to the normal bulbar input (Ketchum and Haberly, 1993). Models of piriform cortex suggest that its networks can, in principle, be made to oscillate at this frequency by way of appropriate and realistic intrinsic recurrent connections (Wilson and Bower, 1992). These data thus suggest that similar cooperative mechanisms may operate in the mammalian and locust olfactory systems.

Conclusions.

In conclusion, local neurons with extensive arbors monosynaptically inhibit projection neurons in the antennal lobe by way of fast, PCT-sensitive, GABA-containing

synapses. This inhibition underlies the synchronization of ensembles of projection neurons and thus the odor-evoked LFP oscillations in the mushroom body. In addition, the odor-specific temporal firing patterns of PNs do not depend on LN-mediated PCT-sensitive inhibition. They may result from slower antennal lobe network dynamics, possibly as a result of different inhibitory mechanisms.

This independence of mechanisms for temporal patterning and synchronization of neural ensembles thus provides a tool to desynchronize odor-coding assemblies without otherwise altering their spatiotemporal composition. Hence, it is now possible to test, physiologically and *in vivo*, the importance of synchronization for odor coding, as shown in the next chapter. Furthermore, it has allowed direct testing of the importance of synchronization in odor perception, learning and memory in a behavioral paradigm (Stopfer et al., 1997).

Special Acknowledgments

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Methods.

Animal preparation.

Adult, non-anesthetized male locusts (*Schistocerca americana*) were taken from a crowded colony and immobilized dorsal side up with their heads attached to a thin vertical glass plate placed under the neck and fixed with beeswax. A watertight beeswax cup was built around the head for saline perfusion. A short piece of polyethylene tubing was placed around the base of one antenna and, to prevent damage from hot wax, covered with a small piece of wiring insulation. The tubing was then waxed into the beeswax cup wall along an axis parallel to the tabletop, leaving the outer 80% of the antenna free for direct airborne odor stimulation. A drop of fast-drying epoxy was placed within the tubing to glue the antenna to the tubing, to prevent movement of the antenna. The epoxy never appeared to make the antenna unhealthy. A window in the cuticle in the dorsal region of the head was cut, sparing one antenna, and the airsacs in the head capsule were carefully removed to expose the brain. The contralateral antennal nerve was cut far from the deutocerebrum, and the antenna removed. The head capsule was superfused with locust physiological saline (in mM: 140 NaCl, 5 KCl, 5 $CaCl_2$, 4 $NaHCO_3$, 1 $MgCl_2$, 6.3 HEPES, pH 7.1) plus 2.5% sucrose at room temperature. The brain was supported by a wax-coated metal platform inserted from the anterior side and also waxed into place onto the cup. In order to maximize stability, the esophagus was transected in the head cavity and the gut removed through the abdomen which was then tied off. After softening with protease (type XIV (Sigma)), the protective sheath was removed from around the brain.

Electrophysiology.

Intracellular recordings were made with conventional sharp glass microelectrodes pulled on a horizontal (Sutter) puller for a DC resistance of 80 to 150 megaohm when filled with 0.5 M K acetate. Recordings were made from the soma or dendrites of local

and projection neurons in the antennal lobe. Antennal lobe neurons could be identified physiologically, since PNs, but not LNs, have overshooting action potentials (sodium spikes) and fire upon depolarization (Laurent and Davidowitz, 1994). Some recordings, however, were confirmed by intracellular fills. Intracellular staining was carried out by iontophoretic injection of cobalt hexamine (6% aqueous solution), with 0.6-s, 0.5- to 2-nA pulses at 1 Hz. Histology and intensification were carried out according to a standard silver intensification process (Bacon and Altman, 1978). Local field potentials were recorded from the mushroom body calyx with glass patch pipettes (1- to 2-mm tips, or 0.5-2 MΩ) filled with locust saline. Stable LFP recordings (as determined by eliciting healthy odor-evoked oscillations) could be achieved for up to 4-5 hours. Data were recorded on Digital Audio Tape (Biologic, France; MDI, New York) for post hoc analysis.

Antagonist application.

GABA (γ-aminobutyric acid) receptor antagonist picrotoxin (PCT) was applied locally to the antennal lobe neuropil by pressure injection with a pico-spritzer, as previously described (MacLeod and Laurent, 1996). Glass capillaries were back-filled with a 0.1M saline solution of PCT and injected volumes were *ca.* 1 picoliter. Controls for absence of PCT leakage outside of the antennal lobe were carried out by dye injection. Controls for absence of mechanical effects of drug injection were done by testing PNs before and after injection of saline in the antennal lobe. In a few PN experiments, PCT was bath applied, which was shown previously to have the same effects on them as pressure-injection.

Odor stimulation.

The open ends of a set (range, 4-7) of Teflon-coated steel tubes (~2 mm outer diameter) were placed ~5 cm from the intact antenna, angled to converge onto the antenna. Each was connected via polyethylene tubing to a sealed capsule containing a filter paper with 1-2 ml of an odorant. Each capsule was connected either independently or serially to

a pneumatic valve system which could deliver timed square pulses of air pressure upon command. Air intake was cleaned and dried via a charcoal and Drierite filter. Odorants were mono-molecular (cineole, citral, isoamyl acetate, pentanol, hexanol, octanol) or blends (cherry, spearmint, strawberry, apple). Odor stimuli were delivered via valves which switched from a constant background stream air to an odor stream of equal flow. A vacuum funnel was placed immediately behind the antenna for rapid odor removal. Odor pulses were 1 second long, with intertrial intervals of 7-10 seconds, delivered in blocks of 7-30 trials per odor. Clean air stimuli were tested to control for mechanosensory sensitivity.

Data Analysis.

Electrophysiological data were digitized post hoc from Digital Audio Tape with LabVIEW software and an NBMIO16L interface (National Instruments, Austin Texas). Thereafter, all analysis was done using MATLAB software (The Mathworks, Natick MA). Local field potentials were digitally bandpass filtered (generally 5-55 Hz) for post-hoc analysis. Sliding cross-correlations were calculated piece-wise over 200-ms-long windows shifterd progressibely in steps of 100 ms over each data set (simultaneous LFP and PN or LN recordings).The cross-correlations in Figs. 3 and 8 were constructed from each pair by averaging over *n* presentations of the odor. Because cross-correlations were constructed on continuous data (LFP and membrane potential), the large variations of potential caused by PN action potential waveforms need to be weighted down for pictorial representation. We thus artificially eliminated all PN action potentials before cross-correlation analysis (spikes clipped to ~5 mV, traces bandpass filtered at 5 to 100 Hz with no phase shift). PN intracellular signals were converted to a list of spike times using a threshold discrimination algorithm. These spike times (rasters) were then used to construct peri-stimulus time histograms over blocks of trials aligned to the odor-pulse command. For analysis of mean firing rates (Fig. 7), the number of spikes was averaged over the odor presentation to

determine the "response" rate, and over the 1 second prior to odor presentation for the "spontaneous" rate.

Chapter 3: The effects of projection neuron desynchronization on the odor responses of downstream neurons.

Introduction.

In the previous chapter, we attempted to define the physiological mechanisms responsible for the synchronization of the neurons in the antennal lobe. Somewhat to our surprise, the critical mechanism – the fast GABA-mediated inhibition of PNs by LNs – turned out to have no obvious role in shaping other aspects of the projection neuron odor responses, such as odor selectivity and slow temporal patterning. This independence suggests that synchrony may play a specific role in olfactory coding, but, more importantly, provides the tool with which to ask the critical functional question: what happens, if anything, to the odor-related information when PN synchronization alone is eliminated from the population activity?

Several studies in mammals have correlated the degree of neural synchronization with specific behavioral or cognitive tasks, such as segmentation, rivalry, and sensorimotor tasks, suggesting a functional link (Roelfsma et al., 1996; Fries et al., 1997; Riehle et al., 1997; Nicolelis et al., 1995). These results, however, do not yet establish a causal link between synchronization and function. Recent electrophysiological and behavioral studies in the honeybee from our laboratory showed that PCT-induced PN desynchronization impairs fine odorant discrimination (Stopfer et al., 1997), although coarse odorant discrimination was spared. Physiological experiments established that similar mechanisms exist in the antennal lobes of honeybees as in locusts for generating odor-evoked synchronization (e.g., PCT-sensitive mushroom body LFP oscillations, PCT-insensitive slow temporal patterning of PN odor responses) (Stopfer et al., 1997). In parallel behavioral experiments, honeybees were conditioned to respond to an odor by the pairing of that odor (conditioned stimulus) with a sucrose reward (unconditioned stimulus). These

honeybees were then tested with the conditioned odor (which represented the control response). In discrimination tests, they were tested with other odors which were either chemically similar or different. Animals whose antennal lobes were treated with PCT were impaired in discriminating the chemically similar test odors from the conditioned odor (fine discrimination), although they were unimpaired in discriminating the chemically different test odors from the conditioned odor (coarse discrimination). These results did establish a causal link between synchronization and perception. They did not, however, address the issue of *how* or *why* synchronized activity leads to improved stimulus discrimination. In other words, the question of where information is lost when PNs— *i.e.*, the information channels— are desynchronized remains to be answered.

In this chapter we present results on a population of neurons downstream from the antennal lobe PNs, the extrinsic neurons of the β-lobe of the mushroom body. The β-lobe neurons were chosen for study because of their location in the olfactory pathway, their clear odor-specific responses, and their accessibility for recording. PNs project to the mushroom body, where they make divergent connections onto *ca.* 50,000 mushroom body intrinsic neurons. While odors cause oscillatory activity in these neurons, they evoke spiking responses only in very sparsely distributed ones (Laurent and Naraghi, 1994), making the sampling of odor-responsive neurons very difficult. We instead focused on the mushroom body extrinsic neurons, which are directly postsynaptic to the intrinsic neurons (Homberg, 1984; Li and Strausfeld, 1997), hence two synapses downstream from the PNs (Fig. 1A). The β-lobe neurons receive convergent input from thousands of mushroom body intrinsic neurons and have clear odor-specific responses (Fig. 2). This makes them a suitable "read-out" of signals processed in the early olfactory system. For reasons of accessibility, the β-lobe was chosen as a target over two other potential sites, the extrinsic neurons of the α-lobe of the mushroom body and neurons of the lateral protocerebral lobe. The α-lobe extrinsic neurons, like those of the β-lobe, receive inputs from MB intrinsic

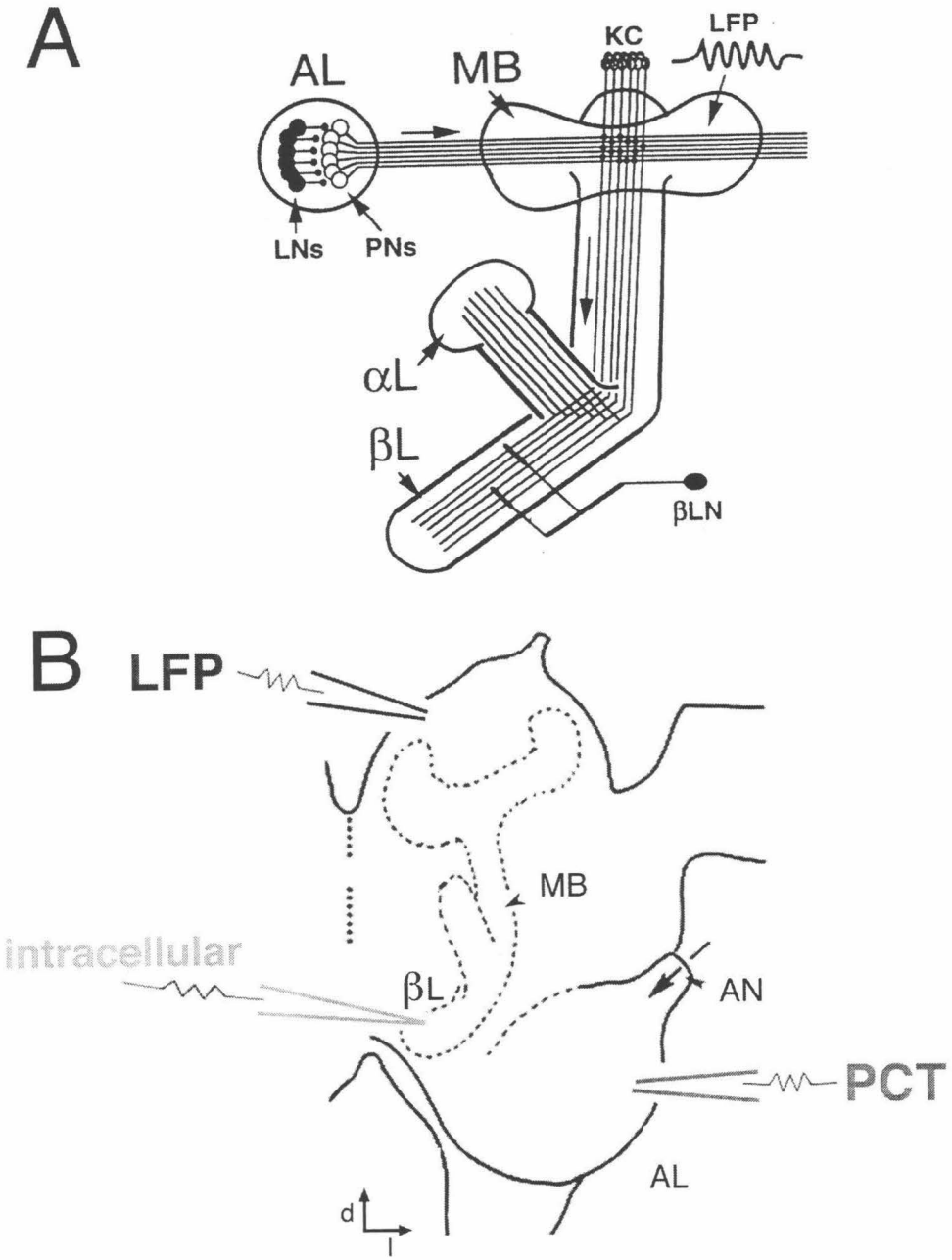

Figure 1. (A) Schematic of the insect olfactory pathways. Antennal lobe (AL) projection neurons (PNs) receive excitatory input from peripheral olfactory receptor neurons and GABA-mediated inhibitory input from local neurons (LNs) (MacLeod & Laurent, 1996; Laurent & Narahi, 1994). PNs send divergent axon collaterals to the mushroom body (MB) calyx, where oscillatory local field potentials (LFPs) can be recorded in response to odours. The intrinsic neurons of the MB (the Kenyon cells, KCs) receive direct excitatory input from PNs (Laurent & Naraghi, 1994; Leitch & Laurent, 1996), and send bifurcating axons to the α- and β-lobes (αL, βL) (Laurent & Naraghi, 1994). βLNs receive convergent input from thousands of KCs, and are thus two synapses downstream from the PNs. (B) Schematic of experimental paradigm. Dendritic recordings of βLNs (intracellular), local field potential reocordings from the calyx (LFP) and local injection of picrotoxin into the antennal lobe (PCT). AN, antennal nerve, d, dorsal, l, lateral.

neurons. The lateral protocerebral lobe is the site of termination for the PN axons after they pass through the MB calyx.

The β-lobe extrinsic neurons (βLNs) are studied physiologically here for the first time in the locust, although extrinsic neurons have also been studied in cockroach (Li and Strausfeld, 1997), honeybee (Homberg, 1984; Gronenberg, 1987; Mauelshagen, 1993), and cricket (Schildberger, 1984). After characterizing βLNs and their responses to odors, we determined the effects of PCT-induced desynchronization of the PNs on these responses.

Results.

Beta lobe neuron morphology.

We recorded intracellularly from the dendrites of these βLNs (n = 37) and filled them when possible (n = 9). These fills confirmed that the recordings were from neurons arborizing in the β-lobe of the mushroom body. Many morphological types were found, indicating a heterogeneous population. Dye injection revealed discrete and dense (presumed dendritic) arbors in the β-lobe and sometimes also within the pedunculus (Fig. 2A,B). Note that these discrete arbors differed from neuron to neuron in the details of their shape and exact position. For example, the neuron in Fig. 2A arborizes in the most proximal portion of the β-lobe, while the neuron in Fig. 2B arborized outside of that section of neuropil. Sparser, varicose (presumed axonal) fibers projected to the α-lobe (Fig. 2A,B,C) and pedunculus (Fig. 2A,B). In some βLNs, very dense arborizations could be seen in the pedunculus (Fig. 2C). The majority of these neurons did not appear to have dendritic or axonal fields outside of the mushroom body. One neuron had dendritic arborizations in the beta lobe, and a dense field of neurites in the neuropil that surrounds the α-lobe, but does not penetrate the α-lobe.

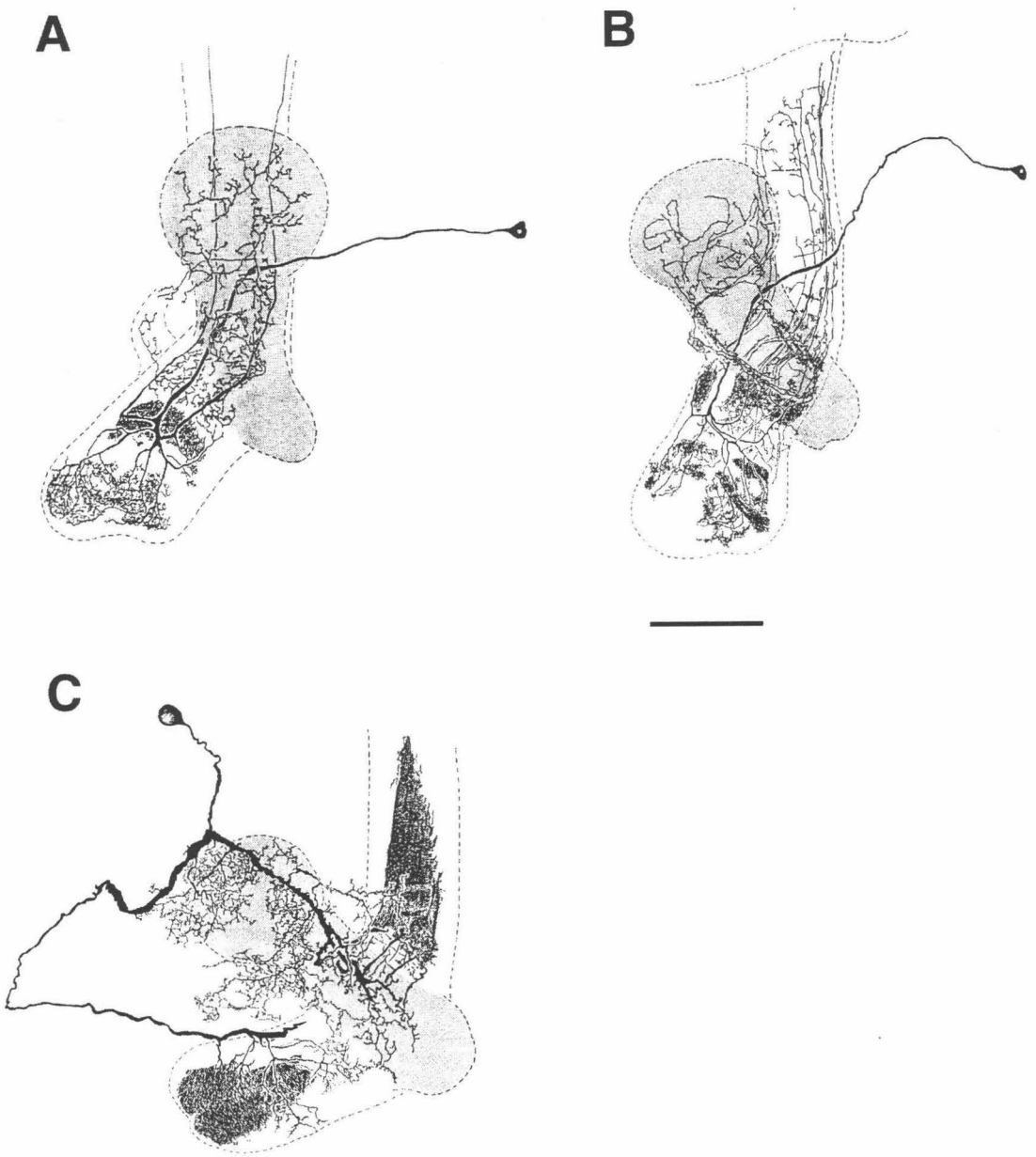

Figure 2. Cobalt hexamine fills of three representative βLNs, each from a different animal. Their odor responses are shown in Figure 3. Calibration, 100 μm.

In some neurons, arborizations in the β-lobe were accompanied by fibers in the lateral protocerebral lobe (not shown). In several fills, the primary dendrite crossed the midline twice before arborizing in the β-lobe. The path of this dendrite might correspond to a tract described in honeybees (Mobbs, 1982) that serves to route extrinsic neurons between the two mushrooms bodies and the protocerebrum (Mauelshagen, 1993). In none of the fills reported here, however, did we see projections to the contralateral mushroom body.

In one case, a Kenyon cell was stained. Thus, for the physiological recordings from which we have no anatomy, we cannot be absolutely certain that all were from βLNs, although KC recordings are highly unlikely. First, due to the small diameter of their axons (100-200 nm), it is unlikely that an axonal recording from a KC would last long enough for the tests described below. Second, the phase relationship of many of the recorded cells relative to the LFP was distinct from that expected of KCs (see below).

The somata of these βLNs were located in at least three different areas in the protocerebrum: the surface of the lateral margin, ventral to the optic lobe attachment; the dorsal midline; the area lateral to the β-lobe itself, in the protocerebrum adjacent to the antennal lobe. Their somata were intermixed with the somata of other, non-mushroom body projecting neurons. Thus, blind somatic recordings from these cell clusters produced a low yield of odor-responsive neurons. We thus opted for dendritic recordings from the β-lobe. When the βLNs were filled from a dendritic impalement, we could often identify a thickened branch likely to be the recording site. This may mean our recordings are biased towards neurons with thick branches in the β-lobe. This may account for our failure to find extrinsic neurons of the types described previously in other species (Homberg, 1984; Mobbs, 1982; Li and Strausfeld, 1997), with flat, laminar, fine arborizations perpendicular to the axis of Kenyon cell fiber bundles, and arborizations in other areas of the protocerebrum. This neuronal type probably exists in the locust also. We also did not find feedback neurons with arborizations in the calyx; in one neuron (Fig. 2A) a few

incompletely filled fibers follow the penduculus toward the calyx, but their terminal arborizations could not be determined. Feedback neurons and fiber tracts between the α-lobe and calyx have been described in other species (Homberg and Erber, 1979; Schildberger, 1981; Mobbs, 1982; Gronenberg, 1987).

Beta lobe neuron physiology and odor responses.

Most of the recordings from the β-lobe could be categorized into two types. The first type was characterized by low spontaneous activity (less than 1 Hz), flat baseline membrane potential with little spontaneous subthreshold activity, and large, narrow action potentials with deep afterhyperpolarizations. (An example is given in Fig. 3A.) The second type was characterized by generally regular, moderate intensity spontaneous activity (up to ~10 Hz), continuous subthreshold activity (often a constant barrage of IPSPs), and action potentials that were broader and of lower amplitude than those of the first type, often with shallower or no afterhyperpolarization. (An example is given in Fig. 10 (top trace).) These two types were recorded with equal frequency.

Similar examples of a morphological type (*e.g.*, Fig. 2A,B) could have different physiological and odor response profiles in different animals, suggesting either many exemplars of a type in each animal, or animal-specific tuning history. Many, though not all, βLNs responded to at least some of the 10 odors that we presented. The neuron in Fig. 3A responded with varying intensities to 4 odors, and negligibly to an air blank. Several neurons also responded to the air blank, and were included in this study only if the response was strongly modulated by the presence of an odor (n = 5).

Responses were usually stimulus-specific but less temporally complex than those of antennal lobe PNs (Laurent et al., 1996). They generally consisted of phasic or phaso-tonic increases in firing rate and, in a few cases, of a suppression of firing (Fig. 3A-C). While spiking responses could be brief, subthreshold synaptic drive consisting of both EPSPs and IPSPs could be maintained throughout the duration of the stimulus and

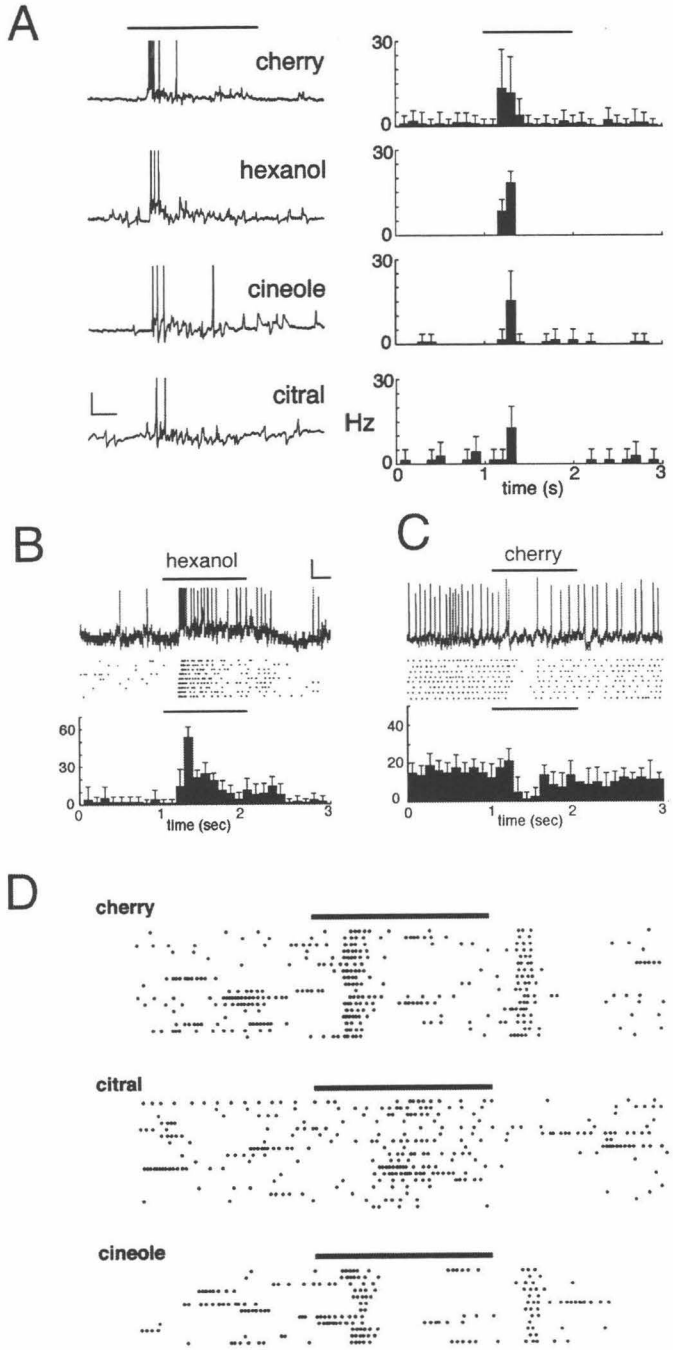

Figure 3. (A) Transient excitatory responses of βLN in Fig. 2A to four different odors. Left, intracellular traces, right, peristimulus time histograms, mean firing±STD. Note subthreshold activity throughout odor stimulus (left). Calibrations, left: vertical, 10 mV; horizontal, 0.2 sec. (B) Prolonged excitatory response of βLN in Fig. 2B to hexanol. Calibrations: vertical, 2 mV; horizontal, 0.2 sec. (C) Suppression of activity of βLN in Fig. 2C in response to cherry. Calibrations: vertical, 5 mV; horizontal, 0.2 sec. For B,C: Top: intracellular trace. Middle: rasters. Bottom: Peri-stimulus-time-histogram, mean firing±STD. (D) Different odors evoke different slow temporal patterns in a fourth βLN. All odor pulses: 1 sec, 7-10 sec between trials.

mushroom body local field potential (LFP) oscillation (Fig. 3A). A few neurons had multiphasic responses which were odor-specific (Fig. 3D). Sometimes these appeared to be a transient early excitatory burst accompanied by a delayed excitatory or an "off" response. Thus, as with the PNs, the βLN temporal patterns were correlated with odor identity, although these patterns were less complex and had greater intertrial variability than those of the PNs.

Phaselocking.

βLN subthreshold activity during an odor response sometimes appeared to be oscillatory. The spikes of the βLNs often phaselocked with the MB LFP oscillation (Fig. 4A). The cross-correlations between the βLN membrane potential and the MB LFP showed a phase-lead of the βLN relative to the LFP by a quarter- to a half-cycle (Fig. 4B). More than half of the sampled βLNs (~63%) showed significant phase-locking of their spikes to the odor-evoked LFP oscillations. For most of the βLNs, the action potential occurred during the trough quartercycle of the LFP. An analysis of this phase-locking is shown in Fig. 4C (for a different βLN from that shown in Fig. 4A,B). The scatter plot displays the time-phase relationship, where each marker represents a spike during one of the 16 trials in response to citral odor (presented from 1 to 2 seconds, horizontal bar at bottom). This neuron had a phaso-tonic firing response (post-stimulus time histogram, bottom) with a tight phase-locking during the odor response. (In this case, the action potentials occurred at ~160 degrees, where the LFP is fitted as a sign wave, peak = 90, trough = 270, and zero-crossings at 0, 180 and 360.) The phase histogram (right) distribution is significantly different from the uniform distribution expected if the spike timing and LFP phase were independent (vertical line). A given βLN responds during a single phase (within 30 degrees) for all odors, and thus odor identity is not encoded by phase of the βLN firing relative to the LFP, a similar result to that shown for PNs (Laurent and Davidowitz, 1994; Laurent et al., 1996; Wehr and Laurent, 1996).

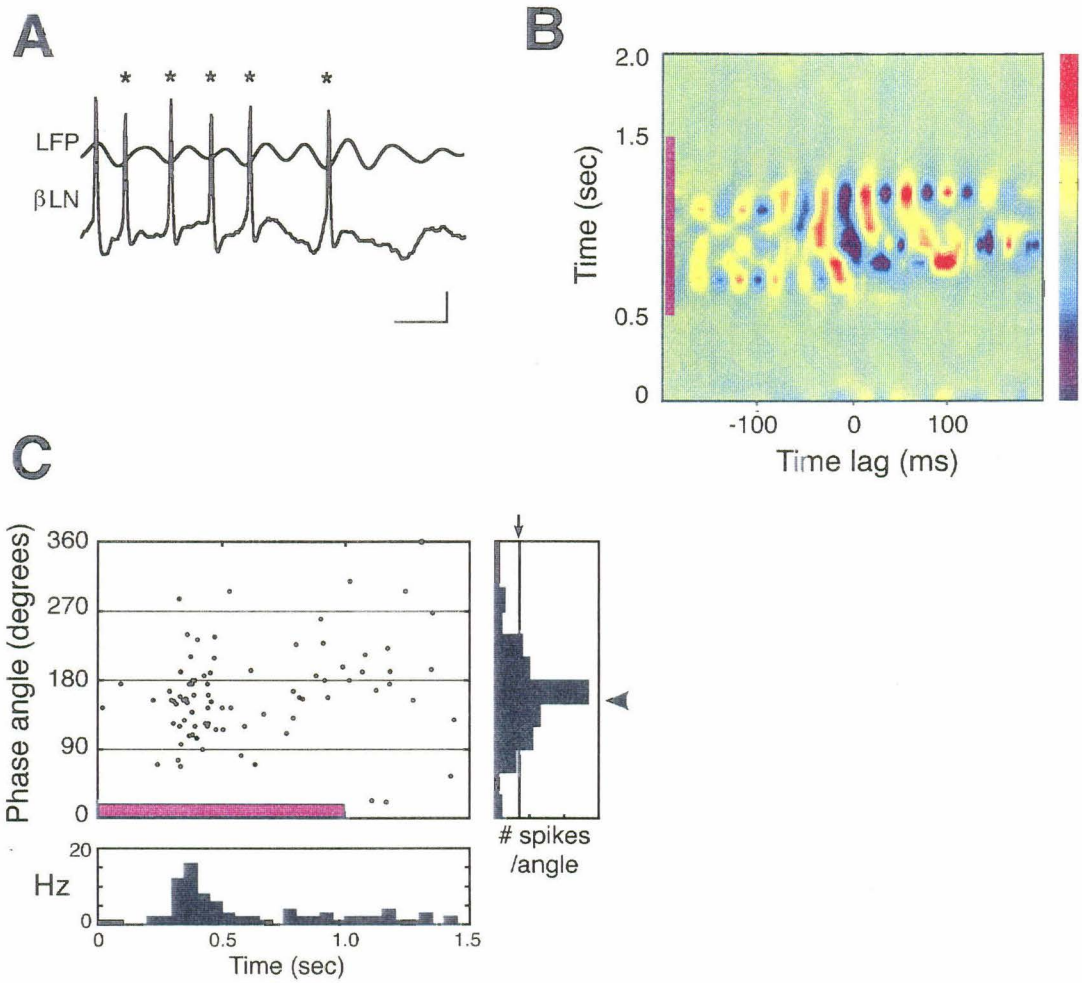

Figure 4. βLNs phaselock with the MB field potential. (A) Spikes of this βLN coincide with the trough of the field potential. Top: LFP. Bottom: intracellular trace. Calibrations: vertical, LFP, 0.5 mV, intracellular, 10 mV; horizontal, 50 ms. (B) Average cross-correllogram of the same βLN as in A, with the MB LFP, over 21 successive trials. Purple vertical bar, 1 second citral stimulus. Color scale: red, positive correlation, blue, negative correlation. (C) Scatter plot shows the distribution of the phase relationship of the action potentials of a βLN relative to the MB LFP oscillation during response to citral odor. Each dot in scatter plot respresents a single action potential, n = 16 trials. X-axis, time (same as bottom); Y-axis, phase of LFP oscillation during which an action potential occurs. LFP oscillation is modelled as a sine wave, with the peak at 90° and trough at 270°. Different neuron than shown in A,B. Bottom: post-stimulus time histogram, mean firing rate. Bin size: 50 ms. Right: Phase histogram shows a peak around 160° (arrowhead; vertical axis, phase angle, horizontal axis, number of spikes per 30° bin). Distribution is significantly different from a uniform distribution (arrow, expected uniform distribution) ($p<0.0001$, Kolmogorov-Smirnov test, Kuiper's statistic).

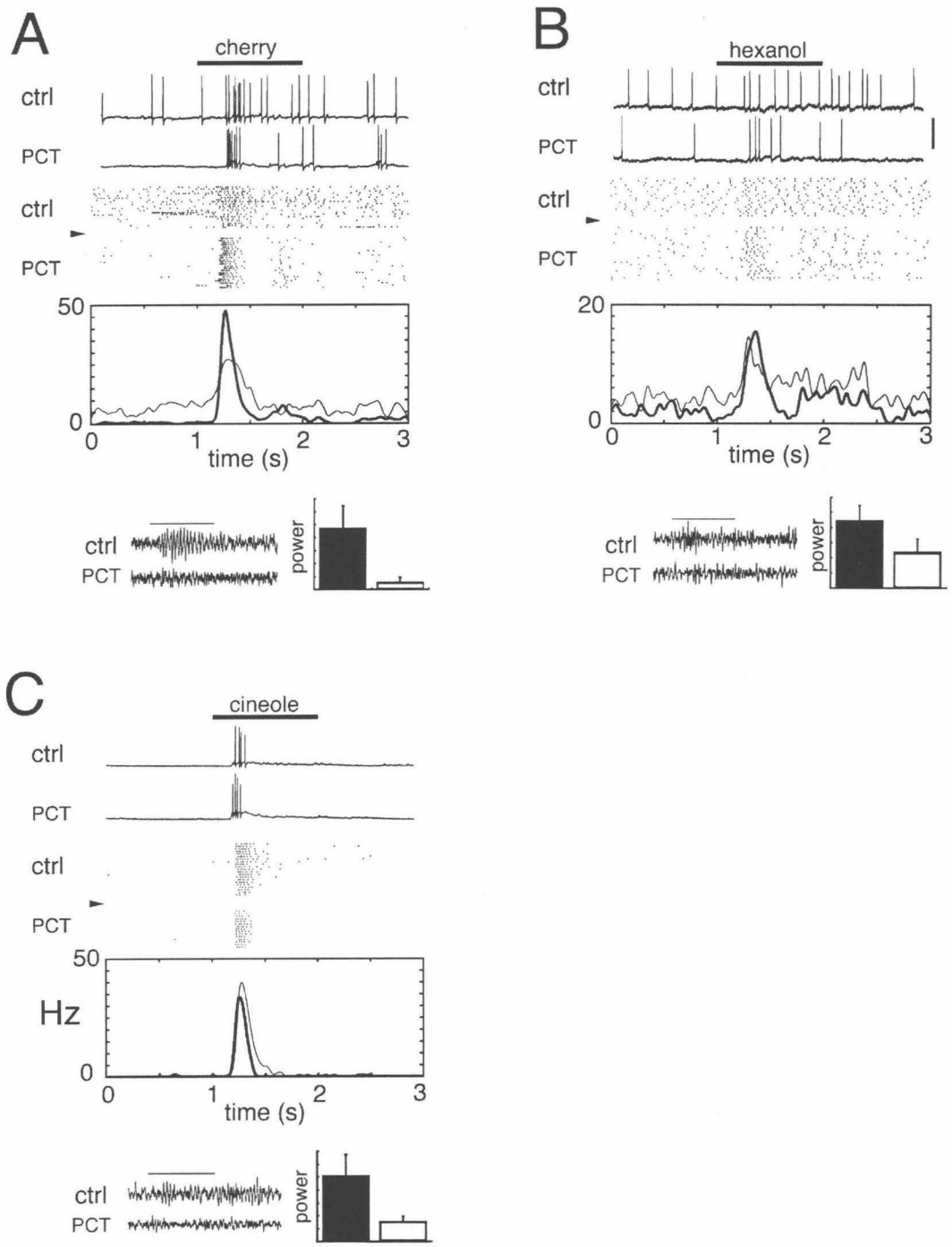

Figure 5. PN desynchronization never suppresses existing βLN odor responses. In these three examples, PCT-induced PN desynchronization has a modeterate (A), mild, (B) or no (C) effect on the responses and background activity. (Top) Intracellular traces before (ctrl) and after (PCT) injection of PCT in the AL. (Middle top) Raster plots of spike times, PCT injection at arrowhead. (Middle bottom) Smoothed PSTH for control (thin line) and PCT (thick line). Odor presented between 1-2 sec. (Bottom) Left, LFP traces. Right, normalized integrated power of LFP in 15 to 30 Hz band, mean ± STD, before (black) and after (open) PCT. Calibration (top), 50 mV.

Did these neurons' responses depend on input synchronization?

Effects of desynchronization on βLN odor-evoked activity.

After characterizing a βLN and its odor responses, we attempted to determine whether these responses were altered in any way by desynchronization of the PNs. In order to do this, we used the antennal lobe picrotoxin injection protocol developed in the previous chapter. A βLN was impaled dendritically and its odor tuning characterized. A local injection of PCT was then made into the ipsilateral antennal lobe (Fig. 1B). PN desynchronization was checked from the spectral analysis of the ipsilateral mushroom body LFPs (e.g., Fig 5, bottom). The βLN responses to odors were then tested again.

We made three main observations on the effects of PCT antennal lobe injection on the odor responses of these βLNs (n=19 βLNs). First, PN desynchronization never suppressed those βLN odor responses that existed prior to PCT injection (Fig. 5-8) (n=15 βLNs).

Second, PN desynchronization often caused changes in existing βLN odor responses (n=14 βLNs) (Fig. 5A,5B,6-8). These changes often consisted of a decrease in baseline firing rates (n=6 βLNs) (Fig. 6), or a combined increase in the response and decrease in the spontaneous firing rates (n=4 βLNs)(Fig. 5A,B), resulting in a more "sculpted" response than in pre-PCT controls. The excitatory bursts in these "sculpted responses" were often shortened and could display higher instantaneous frequencies. The relative response (i.e., normalized to the baseline rate) was therefore often significantly stronger (Fig. 6). In some cases, though, PCT injection had, in fact, no significant measurable effect on βLN odor responses (e.g., Fig. 5C).

PCT injection could cause a change in the temporal patterning of the βLN odor response. For example, an excitatory response could become inhibitory (Fig. 7A), or a weak excitatory response could become multiphasic (Fig. 5A,B; Fig. 7B). Interestingly,

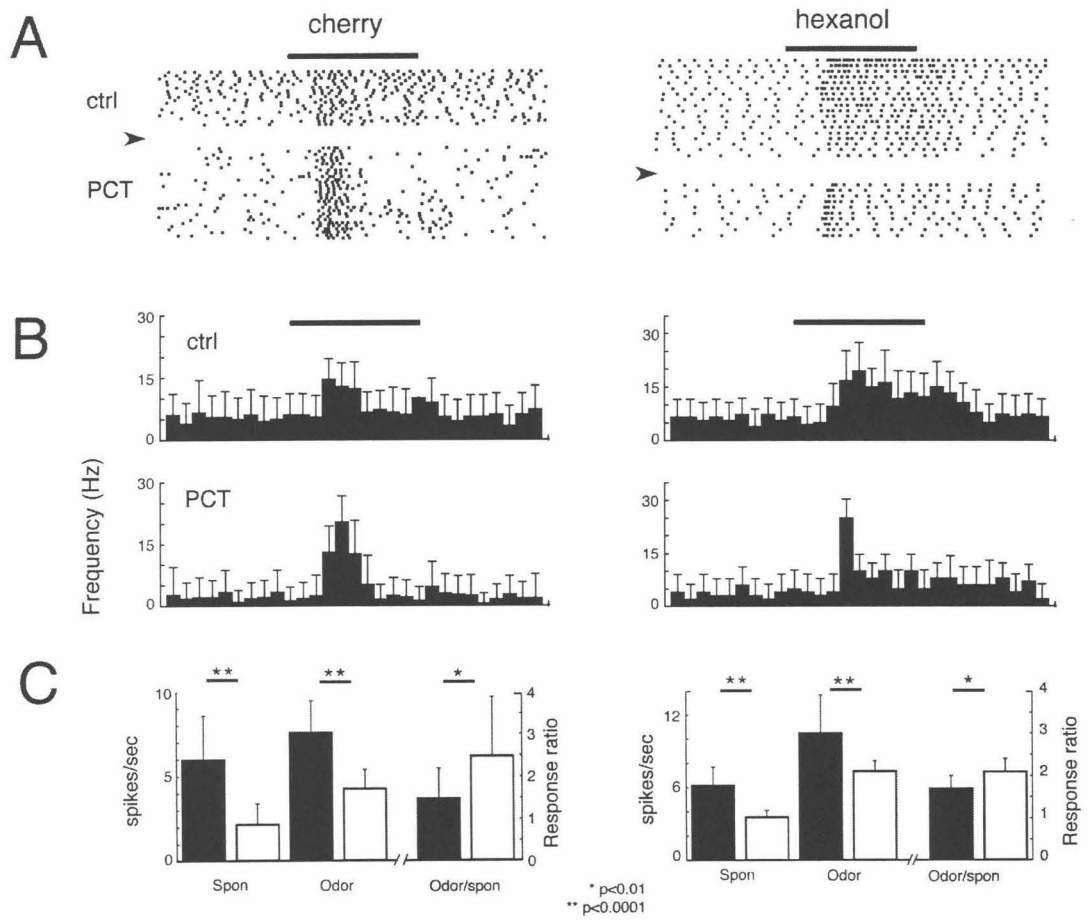

Figure 6. Two examples (A,B) of a decrease spontaneous activity and the resulting odor responses. In each case, the spontaneous activity decreased, and while the odor response activity also decreased, the odor respones relative to the spontaneous activity increased. Top: rasters during control and PCT injection. Middle: PSTH during control and PCT injection. Mean ± std. Bottom: Average activity before odor stimulus (Spon), during and after stimulus (Odor) and the ratio of odor response to spontaneous activity (Odor/spon). Black bars, control; open bars, after PCT injection into AL.

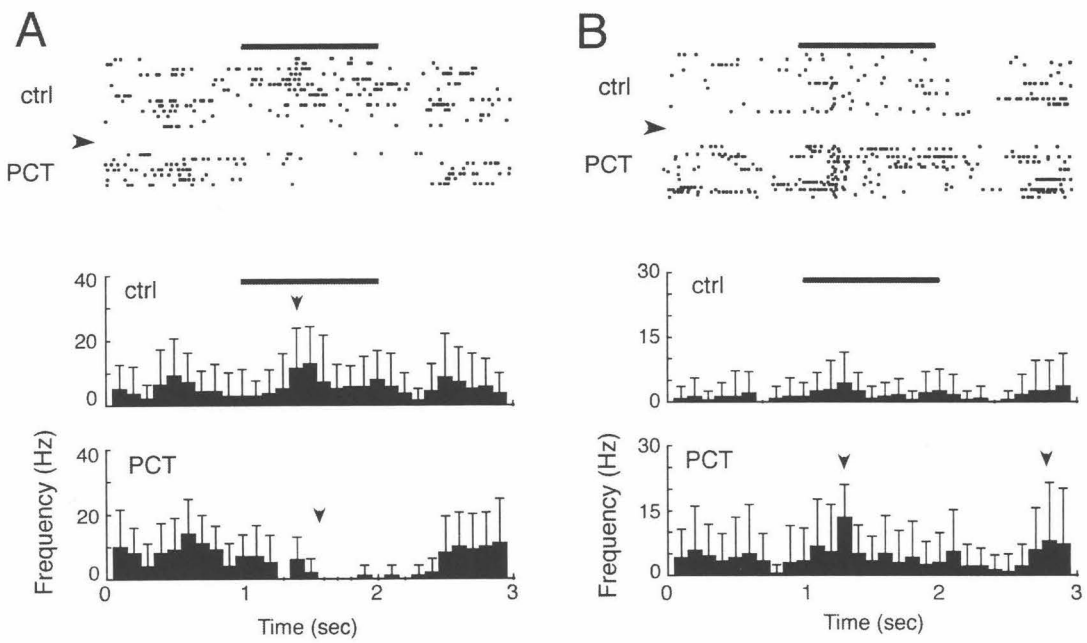

Figure 7. Changes in the slow temporal patterns of two different βLNs. (A) Mild excitatory response become an inhibitory response (arrowheads) after PCT injection into the AL. (B) Only mild excitation, if any, is elicited in control, but a multiphasic response pattern (arrowheads) is clearly elicited after PCT injection. Top, rasters, bottom, peri-stimulus time histograms, before (ctrl) and after PCT injection (PCT).

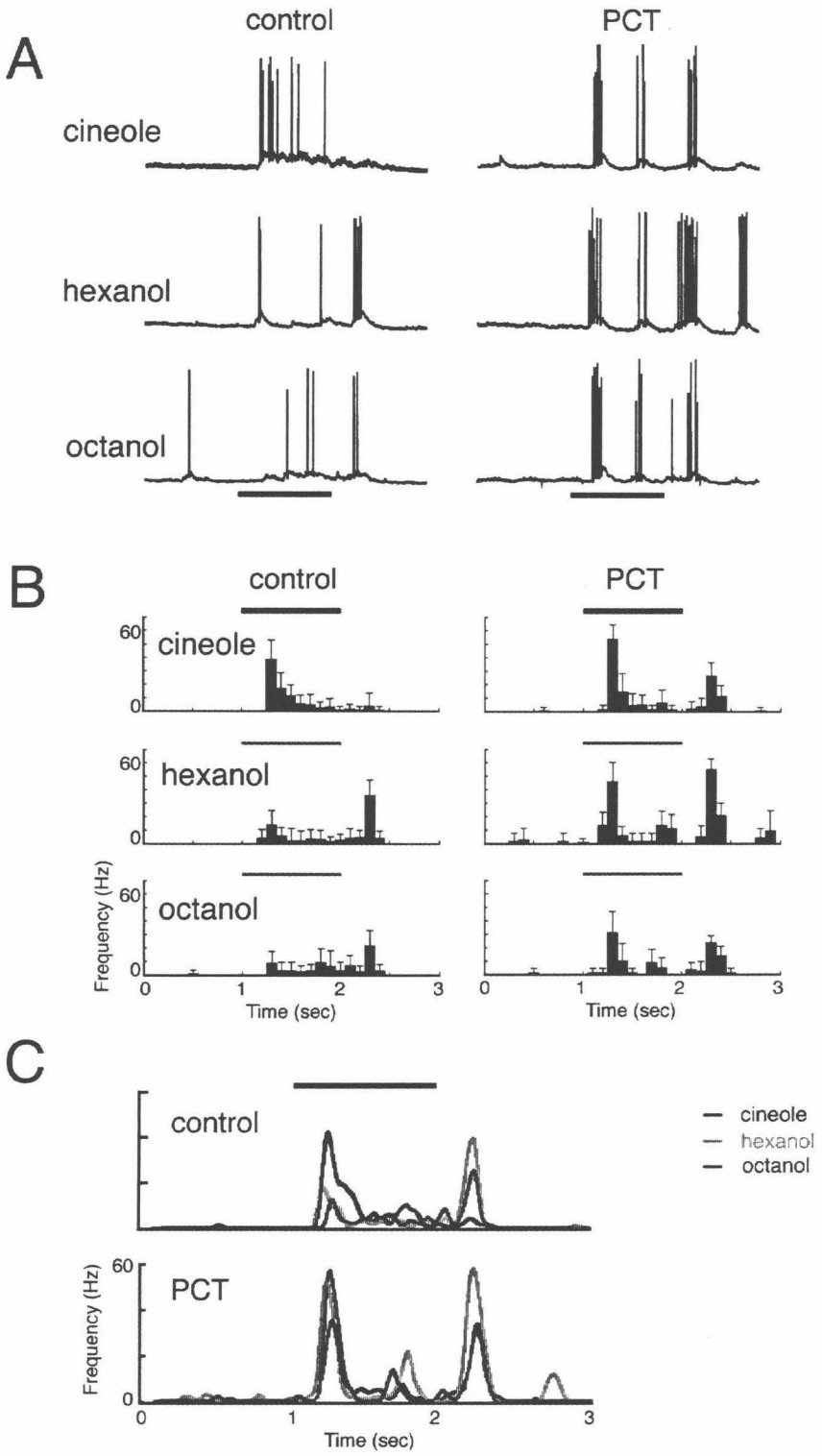

Figure 8. Change in slow temporal patterning of odor responses in one βLN after PN desynchronization. (A) Intracellular traces of responses to three different odors. (B) Peristimulus time histograms for the same odors. mean+std. (C) Comparison of smoothed PSTH's for three different odors (blue: cineole; green: hexanol; red: octanol) before (top) and after (bottom) PCT injection into AL.

these and the above changes often caused responses to different odors to become more similar to one another. An example is given in Fig. 8. Odor responses that were odor-specific became less so after PN desynchronization. These changes will be quantified below.

Induced responses.

Third, PN desynchronization could cause a broadening of odor selectivity, that is, induce a βLN to respond to odors to which it was previously not tuned (Fig. 9-11). One example of this response induction is shown in Fig. 9. In this βLN, the response after PCT is clear not only in the increased firing rate, but also in the membrane depolarization following the cherry stimulus. To quantify such induced responses, we analyzed the data as shown in Fig. 10 (see Methods for parameters). Each set of response trials was rasterized (Fig. 10B), and a smoothed peri-stimulus time histogram was constructed (Fig. 10C). Periods of excitation and suppression were determined (horizontal bars in Fig. 10C); if odor A induced neither excitation nor suppression, the neuron was labelled "non-responsive" to A. Responses were considered "induced" if the βLN was found to be "responsive" after PCT injection into the AL, and if these changes were significantly different in the two conditions during any excitatory or suppressive period (asterisks, Fig. 10C).

Such PCT-induced odor responses occurred in 5 βLNs and in 9 odor-neuron combinations of 14 tested with them. These changes could be odor-specific; i.e., a βLN could acquire a response to one odor and not to another. One neuron, for example, responded to one of three odors in control conditions (hexanol) (Fig. 11A), and after PN desynchronization, acquired a response to octanol (Fig.11B), but not to cineole (Fig. 11C), even though all three odors were clearly effective in eliciting an oscillatory response in the LFP in control trials (Fig. 11, top trace in all three panels). This selectivity was observed in 4 of the 5 βLNs. While most induced responses were simple excitatory response, some

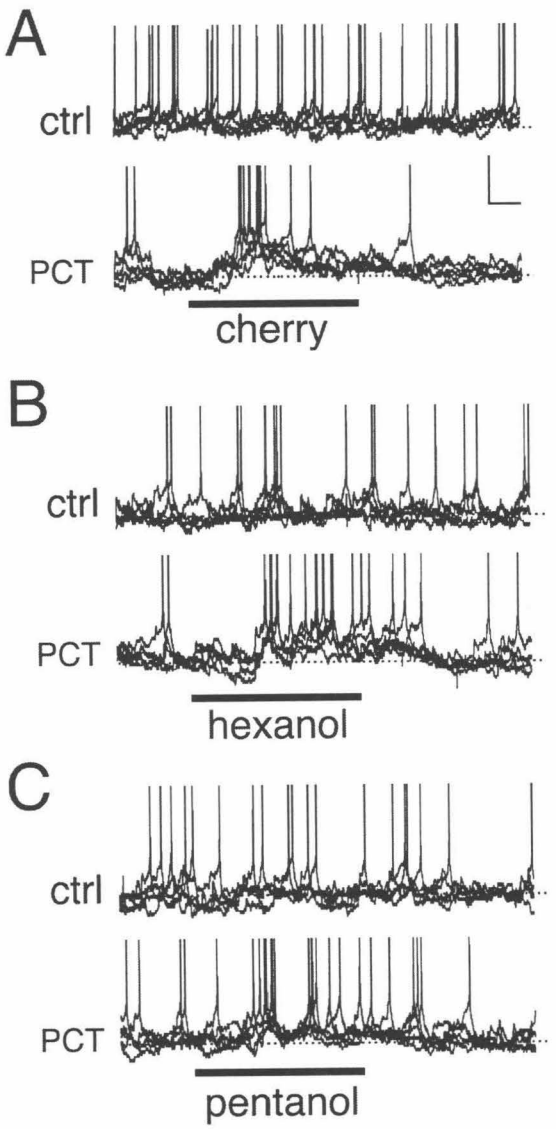

Figure 9. Induction of odor-evoked responses in one βLN after PCT injection into AL. Superimposed intracellular traces from five successive trials, before (ctrl) and after (PCT) PCT injection for three different odors (A-C). Dotted line, resting membrane voltage. Calibrations, vertical, 2 mV, horizontal, 200 ms.

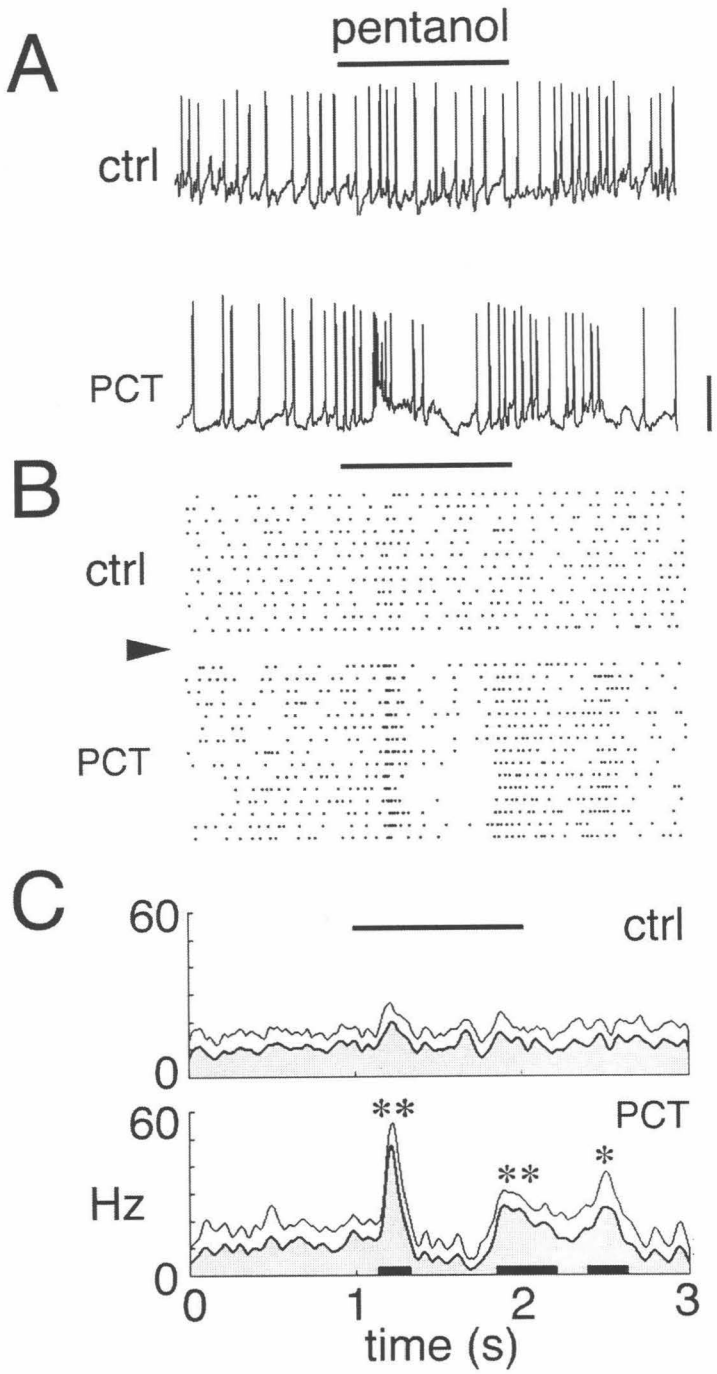

Figure 10. Induction of odor-evoked responses and analysis of significance. Different βLN from Fig. 9. (A) Intracellular traces, (B) rasters, and (C) smoothed peri-stimulus time histogram (PSTH, see Methods). While there was no response to pentanol in control, a multiphasic response to this odor was induced for this βLN after PCT injection. Shaded PSTH represents mean rate, thin line STD. Dark bars at bottom: mean firing rate during epoch delineated by bars is significantly different between control and PCT trials. **: $p<0.01$; *: $p<0.05$.

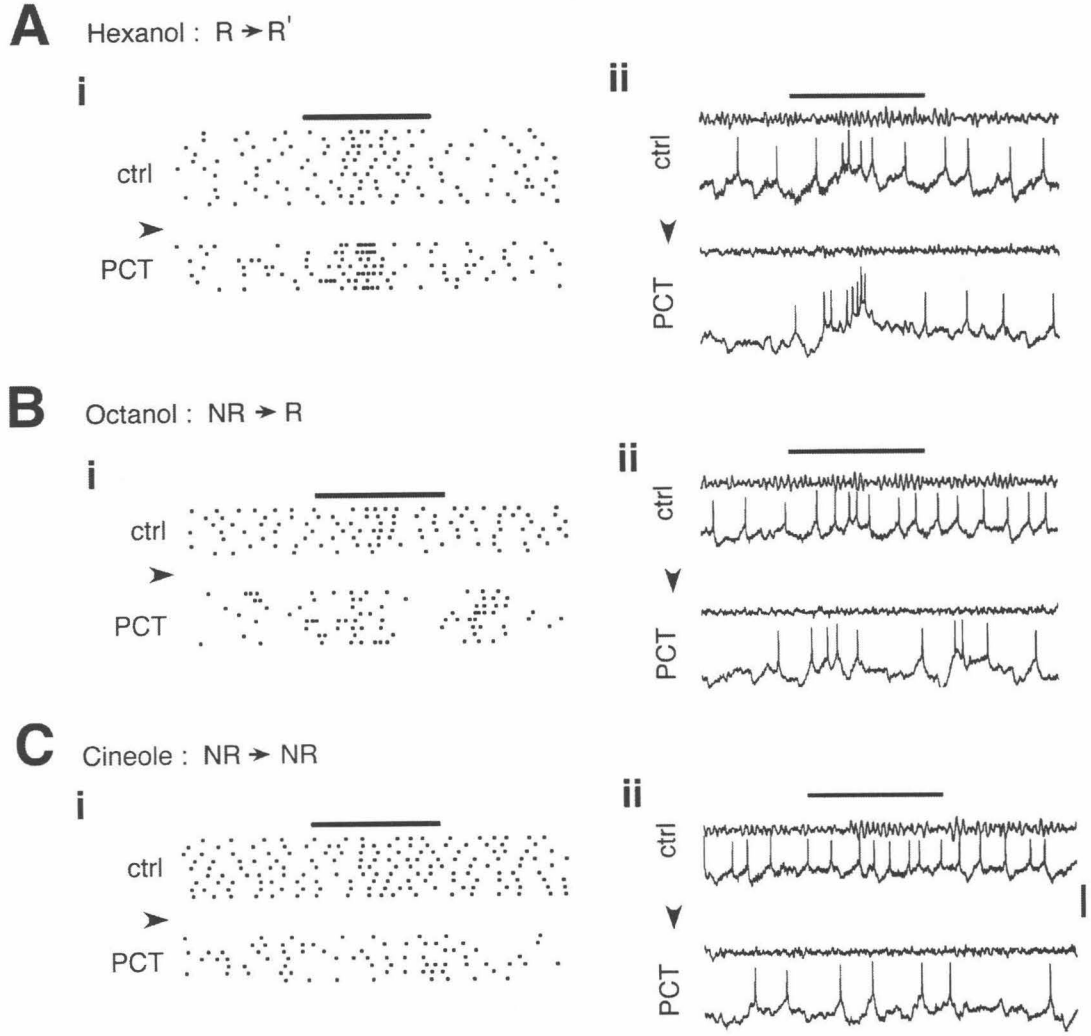

Figure 11. Induction of response is odor specific. In this βLN, during control trials (ctrl), hexanol (A) elicits a response, while octanol (B) and cineole (C) do not. After PCT injection into the AL (PCT), an odor response is induced for octanol (B), but not for cineole (C). The response to hexanol is modified (A, PCT). R, responsive, NR, non-responsive, R', modified responsivity. (i) Raster plots, PCT injection at arrowhead. (ii) Top trace of each pair, MB LFP; bottom trace of each pair, intracellular recording βLN. Calibration, 1 mV (LFP), 5 mV (intracellular). Horizontal bars, odor stimulation, 1 sec.

could be multiphasic (Fig. 11A) or include both excitation and inhibition (Fig. 10). By the same criteria, such PCT-induced responses were *never* observed in antennal lobe projection neurons (n = 10 PNs).

Response stability.

While there was greater inter-trial variability in the responses of the βLNs than those of the PNs, this variability did not seem to indicate trends over long periods of repeated stimuli and responses. Once established, odor-evoked responses were generally maintained over time (Fig. 12). An odor response, if there were one in control trials, appeared within the first 1 to 4 trials of odor testing (i.e., in no case did an odor response simply appear after, for example, 6, 10, 15 trials, etc.). Changing odor stimuli or turning on and off ambient lights did not cause any significant changes in odor responsiveness. Similarly, control injections of saline into the AL cause no changes in βLN odor tuning (Fig. 13). Experiments were discarded if the injection manipulation resulted in a significant worsening of the recording quality. In conclusion, changes in the odor responses following PCT injection into the AL appeared to be caused by PCT injection and not some other concurrent event.

Direct effects of picrotoxin on βLNs.

As with the experiments in the previous chapter, PCT was applied locally to the antennal lobe with pressure injection. Since the beta lobe is in close proximity to the antennal lobe, we were concerned that leakage from the antennal lobe might affect βLNs directly. Three observations suggest that this is unlikely. First, control injections of a dye (cobalt hexamine) into the antennal lobe showed that the injection was well localized to the AL, probably due to the glial boundary separating deuto- and proto-cerebra. Second, we never saw seizures in the LFP recorded in the calyx during any injection experiment, while bath application usually caused seizures. Because seizures are probably caused by direct

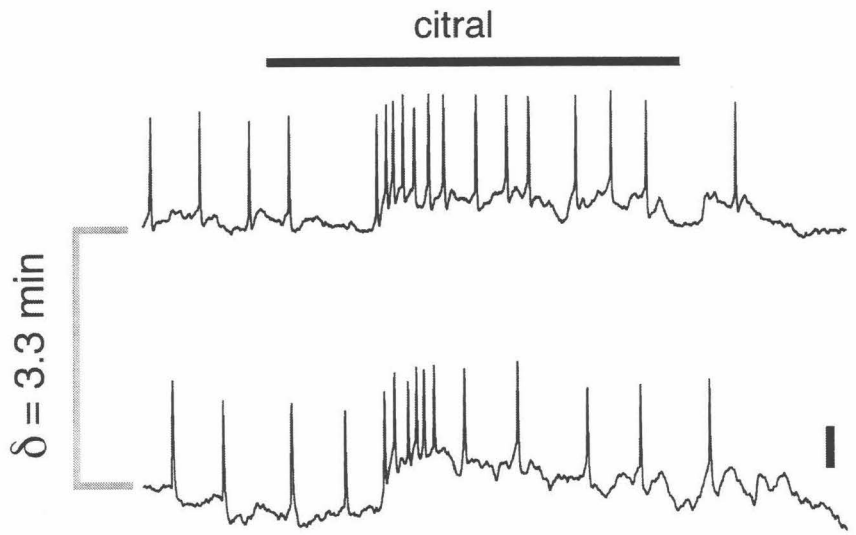

Figure 12. Responses can often be stable over at least several minutes. Here, a βLN response to citral is nearly identical after three minutes of recording and 19 intervening citral trials. Calibration, 20 mV.

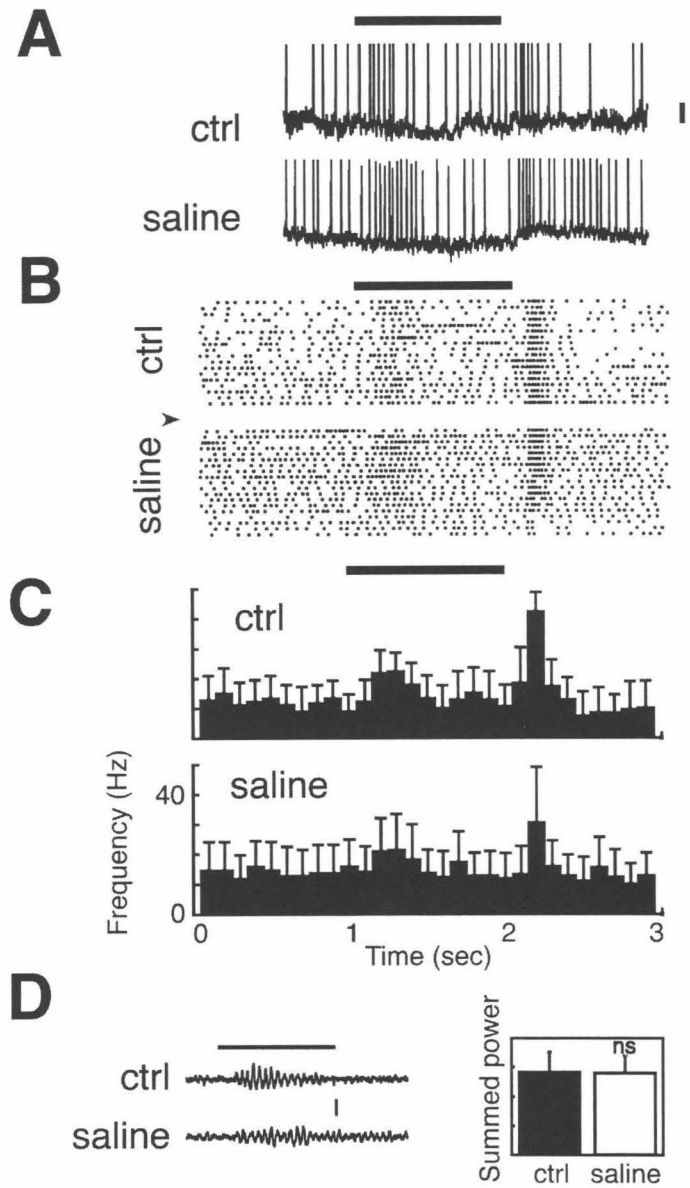

Figure 13. Saline injection does not significantly alter βLN odor responses. Ctrl, pre-injection, saline, after injection of saline solution into the antennal lobe. (A) LFP oscillations and biphasic excitatory response to cherry. Calibrations, 2 mV, 0.4 mV. (B) Spectral analysis of LFP during odor response shows that there is no significant difference (ns) in averaged integrated power over 15-35 Hz band (unpaired *t*-test). Mean+STD. (C) Rasters. (D) Peri-stimulus time histograms, mean+STD.

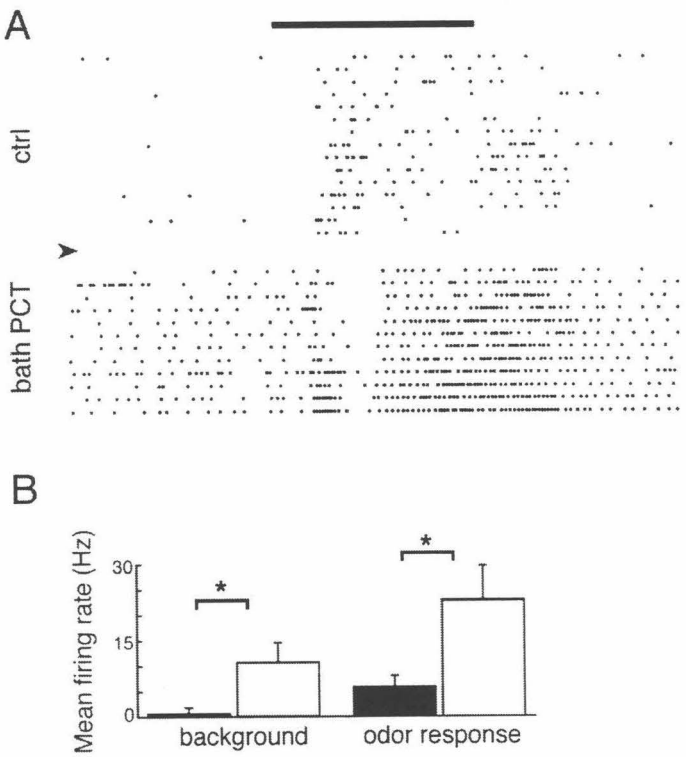

Figure 14. Bath application of PCT generally caused an increase in background, pre-stimulus activity (due to direct activation of the βLN) as well as response activity (due to elimination of synchronization of antennal lobe neurons). (A) Rasters of a βLN during control trials (ctrl) and trials after bath application of PCT (PCT). (B) Comparison of this neuron's firing rate during pre-stimulus period (background) and during odor response, before and after PCT application. Both background activity and odor response increased significantly (* $p<0.01$). Control trials, black bars; PCT trials, white bars.

blockade of inhibition by PCT in the mushroom body, the lack of seizures after PCT injection into the AL suggests minimum PCT leakage into the mushroom body. Third, bath application of PCT generally caused large increases in spontaneous firing in βLNs, while PCT injection often caused a decrease in spontaneous rate (89% of βLN-odor pairs had spontaneous rates that decreased or showed no change).

Quantification of PCT effects on βLN odor responses and comparison with effects on PNs.

The changes we observed in βLN odor responses were notable in that the responses to two different odors often became more similar in PCT than in control trials; this increase in similarity, we hypothesized, might potentially decrease the likelihood of correct odor identification using the information contained in each spike train. To quantify this effect, we used a clustering algorithm to test the effect of PCT treatment on odor classification using βLN responses (Fig. 15). This algorithm categorizes trials and assigns each one to an odor on the basis of similarity between spike trains (see Methods for details).

One example is shown in Fig. 16, where PN desynchronization caused a considerable loss in βLN response specificity to three related aliphatic alcohols. This classification was carried out with pairwise comparisons of all pre-PCT trials (Fig. 16Bi) and indicated 100% discriminability (Fig. 18Ai). PCT was injected into the ipsilateral antennal lobe and classification was repeated, *i.e.*, each response was assigned a probable stimulus given its proximity to any of the three pre-PCT cluster templates. The pairwise (and overall) classification now operated just above chance level (Figs. 16Bii and 18Ai). This indicates that PCT induced both a shift in odor representation by βLNs and a loss of information about stimulus identity in the temporal features of their spike trains.

Over all βLNs for which there was good odor discriminability in pre-PCT controls (n = 14 βLNs, n = 34 odor pairs), PCT injection hindered discrimination of odors (by at least 10 percentage points relative to control responses) in 8 βLNs for at least one odor pair in each neuron and for a total of 19 odor pairs. In 5 of these 8 βLNs, discrimination was

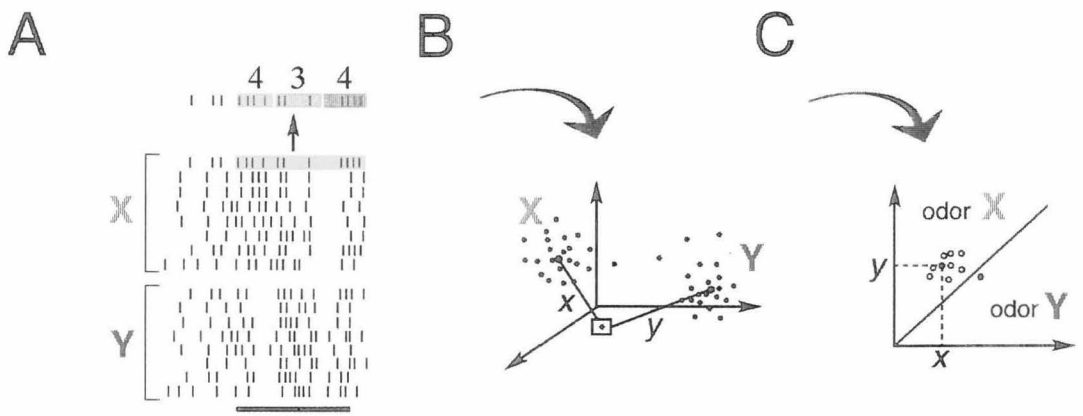

Figure 15. Cluster algorithm. (A) Each spike train in a set (one set for each odor condition (e.g., X or Y) and each pre- and post-PCT condition) was divided into n non-overlapping bins (top) over the post-stimulus onset period. The spike count in each bin was used to construct an n-dimensional vector representing this trial (B). The operation was repeated with each trial in the set, to form a cluster. To assess the separation between two clusters (e.g., for trials of odor X vs. those for odor Y), the Euclidian distance between the point representing each trial and the center of each of the two odor clusters was calculated. For each pairwise odor comparison (e.g., X versus Y), therefore, each trial produced two numbers (e.g., distances to centers of X and Y clusters), that were plotted against each other as one point (C). This trial was then classified by assigning it one likely stimulus, based on to which cluster it was closest (minimum distance). The correct side of the diagonal is that in which the shortest distance corresponds to the odor that was actually presented (e.g., top-left half for odor on bottom axis in C).

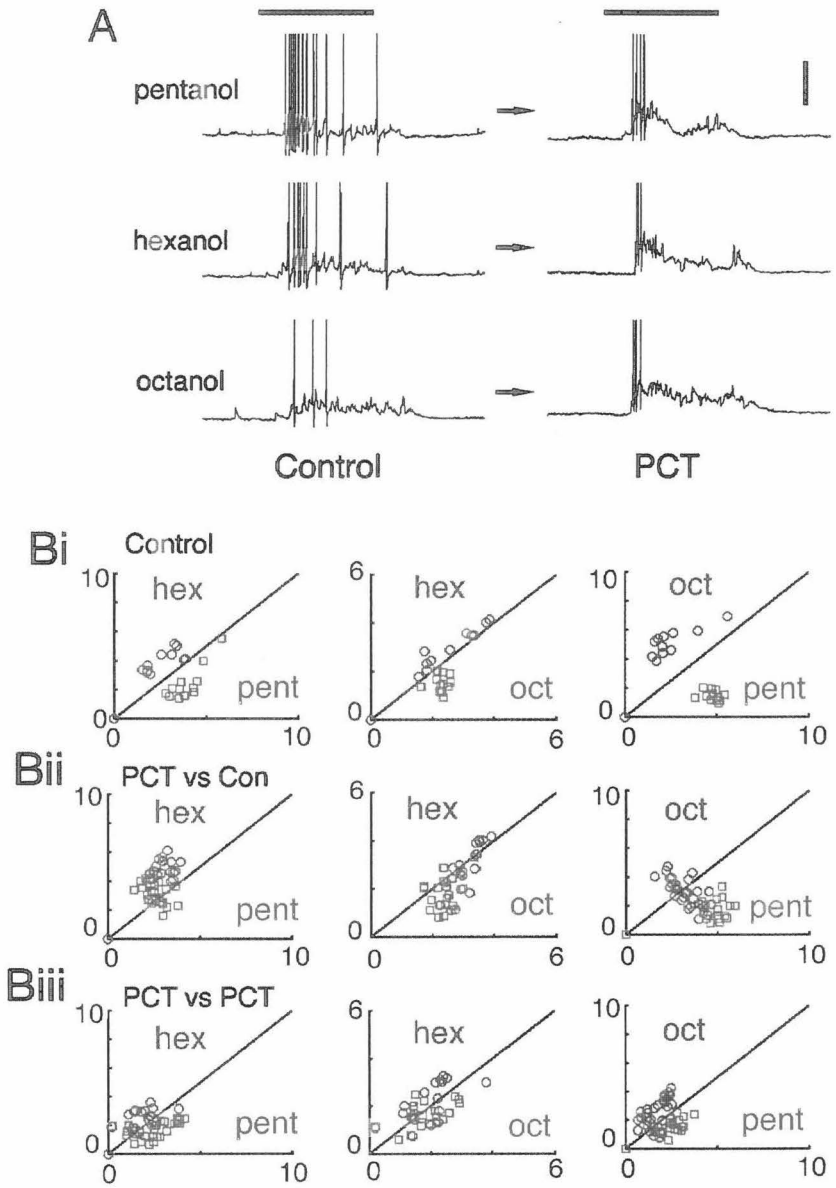

Figure 16. PN desynchronization causes loss of specificity in odor-evoked spike trains in one βLN. (A) Intracellular traces from one βLN in response to three odors before (left) and after (right) PCT treatment. Note decrease in the difference between the three spike trains after PCT treatment. (B) Clustering algorithm (see Methods) applied to control spike trains (i) or post-PCT spike trains (ii, iii) for the βLN in A. (ii) PCT trials are compared to control templates. (iii) PCT trials are compared to other PCT trials. In both cases, PCT trials show an increase in cluster overlap, indicating a loss of specificity. Each point represents one trial. Symbol corresponds to actual odor presented for that trial (e.g., in Bi, leftmost graph: blue corresponds to hexanol, red to pentanol.) x-axis: Euclidean distance between one trial and the mean of all trials for one odor (e.g., hexanol). y-axis: ibid, for a second odor (e.g., pentanol). Diagonal delineates classification, with results shown in Fig. 18Ai.

reduced to chance. Over all βLNs, the percentage of correct *pairwise* odor discrimination dropped from an average of ~90% to ~75% (Fig. 18Bi), suggesting that the PCT- induced changes seen in the βLN responses may explain the behavioral effects observed in honeybees (Stopfer et al., 1997).

To ascertain whether the PCT-induced information loss in βLNs could be attributed to information loss in individual PNs, the same analysis was carried out with the PNs. The one in Fig. 17, for example, responded to three odors with three distinct temporal patterns, allowing 93% correct odor identification on paired choices (Figs. 17A, 17Bi, 18Aii). After PCT injection into the antennal lobe, this PN retained its original odor-specific response patterns, allowing correct odor identification using either pre- or post-PCT templates (Figs. 17A, 17Bii, Biii, 18Aii). Over all PNs that showed above-chance odor discrimination before PCT (16 odor-PN pairs), PCT injection generally failed to alter pairwise odor discrimination using the information contained in these PN spike trains (with a single outlier) (Fig. 18Bii). A different analysis of the spike trains, based on Victor and Purpura's algorithm (Victor and Purpura, 1997), was applied to the same data and confirmed these results (see Discussion, and MacLeod et al., 1998).

A degradation was also found in some βLNs using post-PCT response patterns as templates (*e.g.*, Figs. 16Biii). Such post-PCT discrimination was impaired (decreased by at least 10%) in 5 βLNs (n = 7 odor-neuron pairs), and reduced to chance or by more than 30% in 4 of these 5 βLNs. The same analysis with the PNs (Fig. 17Biii) showed no effect.

Summary

In summary, these results suggest that the desynchronization of PNs alters the βLNs odor responses in such a way that they lose some odor selectivity and specificity. Some changes induced in βLNs by PCT injection consisted of the appearance of *de novo* odor sensitivity, thus broadening the neuron's selectivity. Other changes (in 58% of all neurons and 34% of all odor-neuron pairs tested) were more subtle and consisted of a

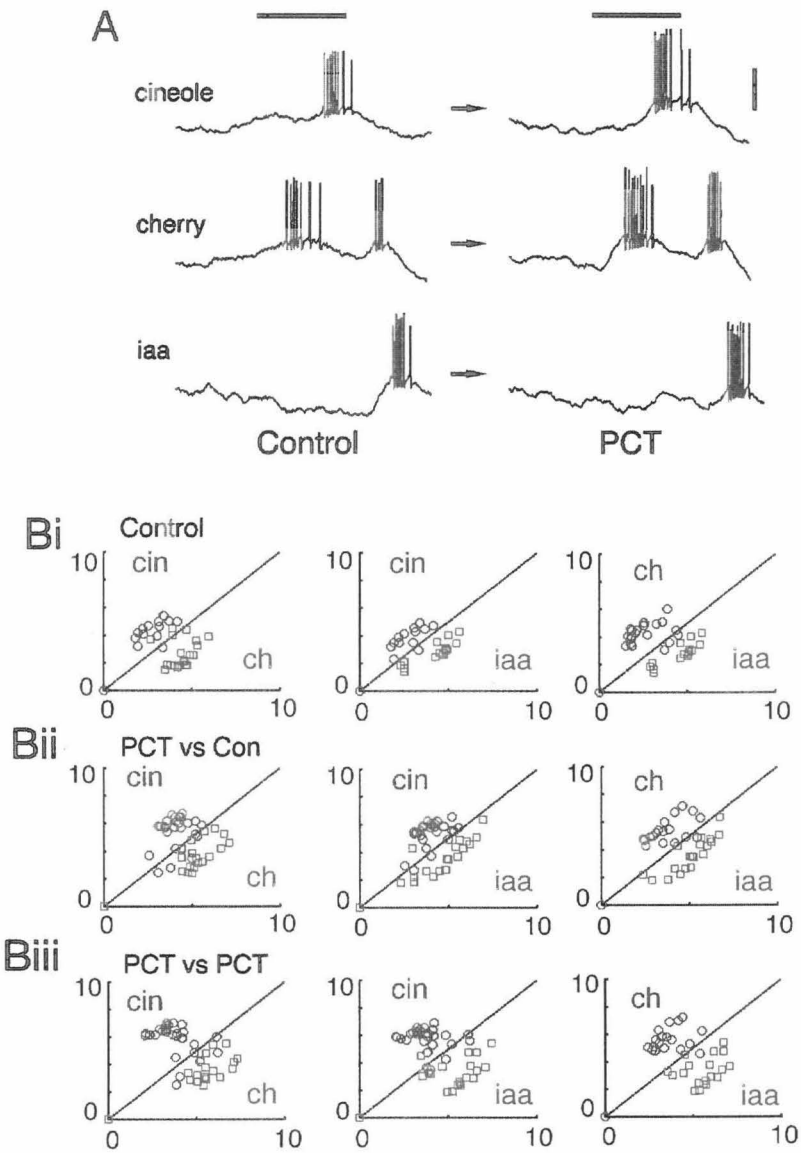

Figure 17. Same as in Fig. 16, but for one PN. (A) Note distinct response patterns to different odors and their persistence after PCT treatment. (B) Same as in Fig. 16B, but for the PN in A. Classification results in Fig. 18Aii.

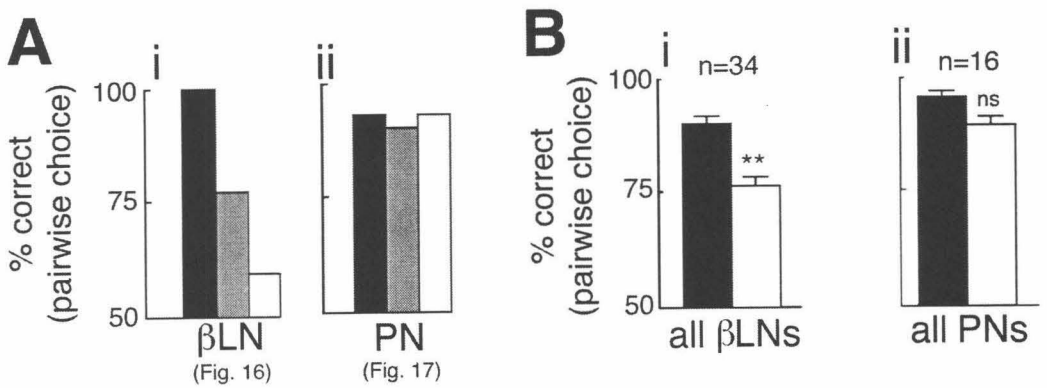

Figure 18. Statistical results on odor classification by βLNs and PNs before and after PCT treatment. (A) Percent correct pairwise odor categorization over all trials for single βLN shown in Fig. 16A (i) and PN shown in Fig. 17A (ii). Black: control vs. control trials; gray: PCT vs. PCT trials; open: PCT vs. control trials. (Ai) βLN shows perfect categorization of control trials (black: 72 correct out of 72) but impaired classification after PCT injection (gray: 147/190 trials; open: 113/190 trials). (Aii) PN shows equally good categorization under all conditions (black: 112/120; gray: 121/134; open: 124/134). (B) Summary of pairwise categorization results for all βLNs (i) and PNs (ii) that showed significant categorization of control trials. Black: control vs. control trials; open: PCT vs. control trials. (Bi) βLNs perform on average with 90% correct pairwise classification in controls, but with a significant decrease in % correct categorization after PCT injection ($p<0.005$, n=34 odors, paired *t*-test). (Bii) PN performance (ca. 95% correct in controls) is not significantly altered (ns, n=16 odors) after PCT treatment.

modification of existing responses to a set of odors. Cluster analysis was used to quantify these modifications. This analysis indicated that the probability of correct odor identification, given a spike train, generally decreased in βLNs after PN desynchronization, but that it remained unchanged in the PNs themselves after the same manipulation. This indicates that βLN responses must depend, in part, on the relational aspects of PN activity.

Discussion.

After PCT-induced desynchronization of PNs, the odor responses of many of the βLNs (58% of all neurons) were changed by either the alteration of existing responses or the induction of *de novo* responses. In no case were odor responses eliminated. For some neuron responses, however, there was no significant effect of PN desynchronization (Fig. 5C). The lack of a measurable effect of PN desynchronization on some βLN responses can be interpreted in two ways. First, the effects of PCT on odor tuning of βLNs were odor-specific. If PCT induced a change in one neuron, it induced that change only for a subset of the odors to which this neuron responded (or did not respond), prior to PCT injection. Our testing of each neuron's odor responses was limited to, at most, 6 odors (a tiny region of all possible odorant space). Therefore, the absence of a desynchronization effect for the set of odors tested cannot be interpreted as an absence of an effect for all odors. It is possible that an effect would have been observed in all βLNs, had we been able to test them with a much larger stimulus set. Second, it is possible that some of the βLNs whose activity we recorded are insensitive to PN synchronization. Dye injections showed that the βLNs are varied in their morphology (Fig. 2), indicating an anatomically heterogeneous population of neurons. The set of βLNs which we studied may thus be also functionally heterogeneous. We have shown in this chapter that more than half of the neurons in our βLN data set are sensitive to PN desynchronization. We cannot rule out, however, that other neurons or neuron types which also arborize in the β-lobe may not be

sensitive to PN desynchronization, and hence that their responses would not be altered by PCT injection into the antennal lobe.

For many βLNs, the odor responses were altered after PCT-induced PN desynchronization, either by an induction or by a modification of odor responses. We have shown that some of these modifications caused, in single βLNs, an increased similarity between the responses to different odors. These modifications were quantified using a clustering analysis in which each spike train in a trial was represented as an *n*-dimensional vector (Figure 15). The analysis presented here classified each spike train of a set based on its similarity to other spike trains elicited by the same odor or to spike trains elicited by a different odor. Thus, a pairwise comparison was made between the sets of spike trains for any two odors for a given neuron. The clustering analysis showed that PN desynchronization induced in the βLNs a shift in the odor representation and a loss of information about the stimulus identity (Fig. 16, 18Bi). No shift or loss of information was found by the identical analysis of the PN data (Fig. 17, 18Bii).

A second analysis was carried out on the same data by applying a clustering algorithm that preserves the continuous structure of the spike trains. This algorithm (Victor and Purpura, 1997) is a cost-based metric according to which a 'distance' is computed between spike trains. This distance is defined as the cost paid to transform one spike train into the other using three steps: insertion, deletion, and displacement of a spike. The set of distances between spike trains were used to assign each one a probable odor stimulus, choosing among all odors presented to a given neuron. Like the first clustering method, the cost-based method indicated a loss of information in the βLN odor responses. The average correct classification over all βLNs dropped significantly from ~68% to ~40% (where chance was calculated to be ~39%) after PN desynchronization (MacLeod et al., 1998). Similarly, no change in the average correct odor classification using PN spikes trains was found after PN desynchronization (control : 61.8%; PCT : 62.1%).

Comparison with honeybee behavioral results.

Behavioral experiments in the honeybee suggest that PN synchronization may have a functional role in fine odor discrimination, i.e., between odors that are chemically similar (Stopfer et al., 1997). Physiological results established that similar synchronizing mechanisms exist in the honeybee and locust antennal lobes (Stopfer et al., 1997). As in locusts, local field potential recordings from the honeybee mushroom body display odor-evoked 20-30 Hz oscillations; honeybee antennal lobe neurons synchronize with one another and phaselock with the LFP oscillations; antennal lobe neurons have distinct slow temporal patterns for different odors; picrotoxin application disrupts the mushroom LFP oscillations but not the slow and odor-specific temporal patterns of the antennal lobe neurons.

In behavioral experiments, honeybees were tested for odor responses after a simple conditioning paradigm. This paradigm consists in the pairing of an odor (the conditioned stimulus) with a sucrose reward (unconditioned stimulus). The unconditioned stimulus elicits the proboscis extension response (PER). Control animals and animals in which PCT was applied to the antennal lobes responded to a high degree to the conditioned stimulus during test trials. Discrimination was tested by presenting, in addition to the conditioned stimulus (e.g., hexanol), test odor stimuli which were either chemically similar (e.g., octanol) or dissimilar (e.g., geraniol) to the conditioned stimulus. In the discrimination tasks, control animals respond significantly less to the test odors (both similar and dissimilar) than to the conditioned odor. PCT-treated animals, however, responded significantly less to the dissimilar test odor than to the conditioned odor, but were as likely to respond to the similar test odor as to the conditioned odor. These results suggested that the PCT-treated animals could not distinguish between similar odors. Thus fine, but not coarse, odor discrimination was impaired.

These behavioral results in honeybee and the physiological results in locust presented here as a consequence of antennal lobe PCT treatment have some obvious

parallels. First, in neither case was there a total loss of olfactory information upon PCT application. In the honeybee behavioral experiments, the animal's ability to detect an odor was not impaired by PCT application. Similarly, in locusts, the βLNs did not stop responding to odors after PCT application. Second, the honeybee's ability to discriminate between dissimilar odors was not significantly impaired, while in locusts, βLN responses continue to be odor-specific after PN desynchronization.

The general finding in locusts (that βLN odor responses lost some specificity upon PN desynchronization) is, therefore, in good agreement with the behavioral results obtained in honeybees, showing that PN desynchronization caused confusion between chemically-related odors. We, of course, do not know if the observed behavioral deficits were caused by the partial de-tuning of β-lobe neurons. The role of βLNs in mediating PER, for example, remains unknown, and βLNs are not the only targets of antennal lobe PNs and Kenyon cells. These results, however, are perfectly consistent with PN synchronization being essential for fine odor discrimination. Because PN responses showed no PCT-induced de-tuning, these results imply that βLNs must construct their response specificity extracting information from the relational features of PN activity. How such decoding, or extraction of information, is achieved is of major interest for the future.

Conclusions.

In conclusion, we have identified a population of locust olfactory neurons located two synapses downstream from the antennal lobe PNs. These βLNs form the first bottleneck after the first two olfactory relays and can be used as a neural "read-out" of population activity in the antennal lobe. The tuning of βLNs was affected by PCT-induced PN desynchronization in two principal ways, *never observed in individual PNs*. First, the specificity of their responses was degraded: odor discrimination using information contained in their odor-evoked spike trains became less likely (often reduced to chance)

after PCT treatment. These effects were odor specific and could affect the responses to related chemical stimuli, blurring their specific differences. Second, PCT-induced PN desynchronization could cause the induction of new, but not generalized, odor responses in βLNs. Because these effects were never observed in PNs, we propose that they were due to PN desynchronization and that βLNs (as well possibly as the neurons presynaptic to them) are sensitive to the temporal structure of their inputs. Our earlier behavioral study indicated that PCT treatment impairs fine, but not coarse, odor discrimination (Stopfer et al. 1997). Our present results may explain these findings. They suggest that behavioral impairments are due to a partial loss of information in β-lobe and/or other neurons equally implicated in the processing of information between early and higher olfactory centers. We thus propose that neural synchronization is indeed important for information processing in the brain: it serves, at least in part, as a temporal substrate for the transmission of information that is contained *across* co-activated neurons (relational code) early in the pathway. Such information—the decoding of which allows enhanced specificity in single downstream neurons—cannot be retrieved by simple averaging of the input channels' activities without considering their temporal relationships.

Special Acknowledgments

The camera lucida drawings in Figure 2 were done by Gilles Laurent. The second clustering algorithm described in the Discussion section of this chapter was done by Alex Bäcker.

Methods.

Electrophysiology, histology and odor stimulation.

Intracellular recordings were made from the dendrites of 37 βLNs by means of glass micropipettes with a DC resistance of 70-130 MΩ when filled with 0.5 M potassium acetate. Intracellular staining was carried out by iontophoretic injection of cobalt hexamine (6% aqueous solution) with 0.6 sec., 0.5-2 nA current pulses at 1 Hz. Histology and intensification were carried out as in (Bacon and Altman, 1977). βLNs may be homologous to multimodal neurons described in other insect species (Homberg, 1984; Li and Strausfeld, 1997; Schildberger, 1984). Local field potentials, intracellular recordings, and pressure injections were carried out as for Chapter 2. Controls for absence of PCT leakage outside of the antennal lobe were carried out by dye injection. Controls for absence of mechanical effects of drug injection were done by testing 8 βLNs before and after injection of saline in the antennal lobe. Bath application of PCT was performed only as control experiments with βLNs. Electrophysiological data were digitized at 5 kHz post hoc from DA Tape with LabVIEW software and an NBMIO16L interface (National Instruments, Austin, TX). All data analyses were performed with MATLAB (Mathworks, Natick, MA). Airborne odors were delivered to an antenna as described in Chapter 2.

Data analysis.

Phase analysis. To determine the phase relationship between the βLNs and the simultaneously recorded local field potential we performed the following analysis. For each trial, βLN intracellular signals were converted to a list of spike times using a threshold discrimination algorithm. At each spike time, the bandpass filtered (5-40 Hz) LFP signal in a 150-ms-window was fitted as a sine wave, and the nearest peaks, troughs, and zero-crossings determined and used as phase reference points: peaks were assigned a phase of

90°; troughs, a phase of 270°; zero-crossings, a phase of 0° or 180°. Phase histograms were constructed with 30° bins. Peaks in the phase histograms were determined to be significantly different from a uniform distribution (expected distribution if there were no phase-locking) by the Kolmogorov-Smirnov test (Kuiper's statistic). Mean phase angles were found by shifting the distribution around the median and calculating the mean.

The following analyses for this chapter were carried out on the βLN data set (n = 19) described here and on the PN data set (n = 26) of Chapter 2.

Test for induced responses. For each set of trials (odor, condition) we calculated a mean±SEM smoothed PSTH by convolving each raster with a 25 msec Gaussian and summing the results. We then calculated, for each time *t* (1 msec resolution), whether the firing rate during the response (2 sec post-odor onset) was different (positive or negative) from the mean baseline firing rate (1 sec pre-odor onset) by at least 2 SEMs for at least 100 contiguous msec. The response was described as different between pre- and post-PCT injection if a significant ($p<0.05$, unpaired two-tailed *t*-test) difference between corresponding epochs occurred. Changes so measured could thus occur over multiple epochs within a response (see Fig. 10C). This method ensured the detection of brief responses or ones that include both excitation and inhibition.

Cluster analysis All βLNs and PNs that responded to two or more of the 10 odors presented were analyzed using a clustering algorithm, illustrated in Fig. 15. Odor discriminations were tested pairwise (*e.g.*, four odors meant six pairwise comparisons). Each spike train in a set (one set for each pre-PCT, post-PCT, and odor condition) was divided into *n* non-overlapping bins over the 2 sec post-stimulus onset period. The spike count in each bin was used to construct an *n*-dimensional vector representing this trial. The operation was repeated with each trial in the set, to form a cluster. We tested bin sizes between 10 and 1800 msec, and found most optimal clustering in most experiments for 50-200 msec, and least optimal clustering below 10 msec (very fine time resolution) or above 1200 msec (approx. average firing rate). We present data for 100 msec bins, and

thus $n = 20$. The object was to assess the separation between two clusters (*e.g.*, the pentanol cluster *vs.* the hexanol cluster), by assigning each spike train (or vector) one likely stimulus on the basis of its Euclidean distance to the center of each of the two clusters. For each pairwise comparison, therefore, each trial produced two numbers (*e.g.*, distances to centers of pentanol and hexanol clusters), that were plotted against each other as one point. The percentage of correct classification over all trials was given by the proportion of points that fell on the "correct" side of the diagonal. The correct side of the diagonal is that in which the shortest distance corresponds to the odor that was actually presented. Significance of the "percent correct classification" was calculated by finding its binomial probability, given a forced choice (threshold $p<0.05$; paired one-tailed *t*-tests). Control trials were compared (1) to control trials and (2) to PCT trials. PCT trials were compared to PCT trials (3). Comparison (1) describes classification of odor responses in control conditions. Comparison (2) describes classification of post-PCT odor responses based on their control templates. This comparison best assesses the information loss caused by input desynchronization. Comparison (3) describes classification of post-PCT odor responses based on their post-PCT templates.

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