INTRAGLOMERULAR COMMUNICATION IN THE MAMMALIAN OLFACTORY BULB

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Declaration:

I, Diogo de Oliveira Pimentel, confirm that the work presented in this thesis is my own. This work has resulted in a publication authored by myself and my supervisor Dr. Troy Margrie (Pimentel and Margrie 2008; J Physiol **586**(8): 2107-19), a copy of which is annexed to this thesis.

Abstract:

The olfactory bulb (OB) is the first processing structure in the olfactory system; it receives direct sensory input from the olfactory sensory neurons and relays processed information to the olfactory cortex and other structures in the brain. The axons from sensory neurons expressing the same olfactory receptor molecule converge onto the same discrete structure on the surface of the OB, termed glomerulus. Each glomerulus forms a modular unit which confines the single apical tufts of about 25 mitral cells (MC). Understanding how intraglomerular cells communicate with one another will therefore improve our understanding of how the glomerular network processes the olfactory signal.

Using whole cell recordings from mitral cells in acute mouse olfactory bulb slices I have examined various modes of MC-MC communication. MC dendrites are known to release glutamate from their apical and lateral dendrites which has been shown to depolarize their own presynaptic dendrites (self excitation; SE). I first examined the anatomical locus of SE by investigating its properties in intact versus dendrotomized (those with amputated tufts) MCs. While dendrotomized cells lacked detectable self excitatory potentials I have found that all morphologically identified MCs with an intact apical tuft displayed robust SE. This form of SE was mediated by Ca^{2+} permeable AMPA receptors as it was completely blocked by their specific antagonist Naphthyl-acethyl-spermine (NAS).

Electrical coupling between MCs was assessed by injecting large hyperpolarizing pulses. I have found that MCs that shared the same glomerulus were always bidirectionally coupled via gap junctions though the magnitude of coupling (CC) was variable across different pairs.

Action potentials evoked in one MC also results in EPSP-like depolarizations in intraglomerular partners (lateral excitation; LE). In contrast to the previously

mentioned forms of communication LE was not reliably present in all intraglomerular pairs. I have found LE is mainly a unidirectional form of communication, although both bidirectional and complete lack of measurable LE can also occur. The magnitude of LE varied independently of that of other forms of communication (CC and SE amplitude) as well as with morphometric characteristics of the apical tufts (number of nodes, surface area). Despite being dependent on glutamate receptors LE contrasted with SE in that it was insensitive to NAS and therefore is a distinct form of chemical communication that relies on different AMPA receptor subtypes.
I have also investigated whether in vivo-relevant patterns of theta-burst stimulation (TBS) could evoke significant long-term changes in LE efficacy similar to those observed at axo-dendritic connections. This form of communication showed a unique sensitivity to TBS in that the polarity and amplitude of change in efficacy was linearly and inversely correlated with the initial strength of LE. Small connections were enhanced while larger ones depressed, the change in efficacy was accompanied with a change in paired pulse ratio consistent with a presynaptic change in release probability after TBS.

All together these data show that SE, electrical coupling and LE are independent forms of intraglomerular communication which might serve independent roles on glomerular processing. While SE and electrical coupling have been shown to facilitate spike synchrony across the network, LE provides the OB with a means of modulating intraglomerular excitation in response to in vivo -relevant patterns of activity.

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Abreviations:

a.u	arbitrary unit
aCSF	artificial cerebrospinal fluid
AHP	afterhyperpolarization
AMPA	a-amino-3-hydroxy-5-methyl-4-isoxaleproprionate
AP (s)	action potential (s)
AP-V	R-2-amino-5-phosphonopentanoate
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
CC	coupling coeficient
CH	convex Hull
CNC	central nervous system
Con.	Control
CP- AMPA	calcium permeable AMPA receptor
CV	coeficient of variation
EPL	external plexiform layer
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
GABA	g-aminobutyric acid
GC	granule cell
GCL	granule cell layer
GL	glomerulus layer
GTP	guanosine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
inj.	injected
IPSP	inhibitory post synaptic potential
IR-DIC	infra-red diferential interference contrast
JC	juxtaglomerular cell

LE	lateral excitation
LOT	lateral olfactory tract
MC	mitral cell
MCL	mitral cell layer
NAS	Naphthyl-acethyl-spermine
NBQX	3-dihydroxy-6-nitro-7-sulfamoyl-benzo (f) quinoxaline
NMDA	n-methyl- D-aspartate
Norm.	normalized
ON	olfactory nerve
OR	olfactory receptor
ORN	olfactory receptor neuron
P	postnatal day
PG	periglomerular cell
PPR	paired pulse ratio
PSP	postsynaptic potential
-R	receptor
rec.	recorded
rIPSP	recurent inhibitory post synaptic potential
Rm	membrane input resistance
SE	self excitation
TBS	theta burst stimulation
Vm	membrane potential
Vrest	resting membrane potential

Chapter 1 Introduction:

Neural communication in the nervous system

In order to allow the flow and processing of information in the brain, neurons have to communicate between each other within the network they are part of and also between different networks. This is necessary for the brain to integrate sensory information and then instruct the efferent structures (muscles, organs etc) with the computed output. Thus information arising in sensory neurons (for example olfactory sensory neurons) has to be relayed to the relevant processing centers where the appropriate response is determined to instruct an action.

Neurons are highly specialized cells with a very complex morphological structure. They communicate with their partners via anatomical specializations which are generally called synapses. Two distinct contributing elements can be considered in such a form of communication: the presynaptic element, the one transmitting the information and the postsynaptic element or target, the one receiving information. It is the strikingly different mechanisms and machinery underlying information flow between these two elements in neural networks that subdivides synapses into two main groups: the electrical synapses and the chemical synapses (Bennett & Zukin, 2004). The difference between the two is rather simple; while electrical synapses rely on the physical continuity of the cytoplasm of both cells, chemical synapses have a physical gap between the two cellular membranes which is bridged by the release of a chemical transmitter (the neurotransmiter) by one cell (presynaptic) and detected by the reception machinery in the second cell (postsynaptic). Historically these two mechanisms were first proposed as alternatives to each other. Initially neuroscientists debated between which of those would actually occur given that both could explain most observations and requirements thought to underlie neural communication (Eccles, 1982; Bennett, 2000a). Although great initial attention has

been provided to understanding chemical transmission which was thought to play a more dominant role in neural networks it is now accepted that both forms co-exist in mature neural systems in which they play complementary roles in neural processing (Bennett & Zukin, 2004; Hormuzdi *et al.*, 2004).

Electrical transmission in the nervous system

Electrical synapses are perhaps the less studied version when compared to the extensive studies that have aided the understanding of chemical transmission. Historically chemical synapses were given a more prominent role due to their relative abundance in mammalian systems (Bennett & Zukin, 2004). The finding that functional electrical synapses are widespread in several regions of the adult brain and responsible for features of these networks which are thought to be critical for processing has reignited the enthusiasm to understand the function and specification of these structures (Hormuzdi *et al.*, 2004).

The term, electrical synapse, refers to a form of communication which relies on an anatomical specialization called a gap junction. The gap junction consists of a region in which the membranes of both cells are very closely apposed and pierced together by a battery of pores that allow the flow of ions, and thus electrical current, between the two. Such pores will allow not only the flux of ions, which accounts for electrical coupling, but also the diffusion of small molecules and metabolites allowing for the coupling of non electrical forms of modulation. This form of communication contrasts with chemical transmission in that it does not rely on a given neurotransmitter molecule and postsynaptic reception machinery but rather on the direct "electrical continuity" of the coupled cells and thus permeant ions carry out the transfer of the electrical signal (Bennett & Zukin, 2004; Hormuzdi *et al.*, 2004; Sohl *et al.*, 2005). These pores function as a leak conductance which allows a more depolarized cell to excite a less depolarized partner but the opposite can also occur leading to a hyperpolarization of the postsynaptic element. Thus the same

synapse can be both excitatory and inhibitory depending on the excitatory/inhibitory drive into each cell. Further, the two cells are electrically coupled in a bidirectional way as ionic flow can usually occur in both directions (unless perhaps in very extreme cases of very large differences in input resistance of the coupled cells or incorporation of rectifying subunits). These features allow for rapid transfer of subthreshold activity between cells acting as normalizing agent balancing excitation and inhibition of interconnected ensembles rather than committing to either of the forms. Such feature also contrasts with fast chemical transmission, which in general transmits information in a fixed direction (from releasing element to receiving element) and in that chemical synapses are either excitatory or inhibitory.

The fact that gap junctions function by the means of membrane pores means that the biochemical identity of the cell is to some extent compromised. The permeability of gap junctions is not such that they allow the complete mixture of intracellular contents but nevertheless allows the interchange of small metabolites and signaling molecules such as cyclic Adenosine monophosphate (cAMP) and inositol triphosphate (IP_3) which are known key players in several intracellular signaling cascades . This way intracellular signals that otherwise would be confined within the membrane's biochemical barrier of each cell can be bridged allowing modulation between different cells. Among such metabolites are well known modulators of neurotransmitter release and reception across neuronal membranes but which have also been shown to modulate and/or gate gap junction conductance itself (Bennett & Zukin, 2004; Hormuzdi *et al.*, 2004; Herve & Sarrouilhe, 2006). This possibility might well extend the benefits of intracellular signaling pathways to more cells than those immediately involved in the direct detection of an extracellular signal.

The building blocks for gap junctions are the connexins (Beyer *et al.*, 1990), or the less abundant pannexin (Bruzzone *et al.*, 2003), transmembrane proteins. The connexin protein family is a large multigene family with aproximately 20 identified members in the mammalian genome. Single connexin proteins arrange themselves

in hexamers to form a hemichannel, which is organized in a way that a pore in the membrane is formed surrounded by the hexameric assembly (Connors & Long, 2004). Each of the interconnected cells contributes to the formation of the gap junction channel with one hemichannel. The stoichiometry is 1:1, each hemichannel in one cell connects to another in a second cell holding the two membranes in very close proximity and forming the pore that connects both cytoplasms but nevertheless does allow leak to/from the extracellular medium. Hemichannels can be homomeric or heteromeric and depending on the subunit composition the junctional conductance, rectification, gating, and even mechanisms for modulation of conductance will vary (Connors & Long, 2004). Connexins have multiple putative phosphorylation sites some of which have been shown to be targets for protein kinases thought to be involved in the gating/modulation of these structures (Hormuzdi *et al.*, 2004; Landisman & Connors, 2005; Sohl *et al.*, 2005). Although often the view on gap junction mediated coupling is of a generally hard wired passive conductance, several studies have shown that, and in particular in the context of neuronal gap junctions, the connection can be modulated (Landisman & Connors, 2005). In the nucleus accumbens, a sub region of the striatum, it is thought that modulation can occur as a direct consequence of dopaminergic input. In this structure D1 receptors have been shown to decrease dye coupling while D2 receptors have the opposite effect (O'Donnell & Grace, 1993). Similar dopamine dependent modulation of junctional conductance has also been well studied at the retina (McMahon *et al.*, 1989; Harsanyi & Mangel, 1992; O'Donnell & Grace, 1993; Hormuzdi *et al.*, 2004).

Electrical synapses are often regarded as faster signal transmiters than chemical synapses and this is often considered as one benefit over chemical transmission. Indeed there is an inherent delay necessary for release and diffusion of the neurotransmitter molecule that does not exist for electrical synapses. However in warm-blooded animals the latency difference is small given that body temperature

reduces diffusion times (Bennett, 2000b; Bennett & Zukin, 2004; Connors & Long, 2004; Hormuzdi *et al.*, 2004).

Electrical synapses are more reliable, bi-directional and as mentioned above can have both excitatory and inhibitory effects. Excitation of a postsynaptic cell is at the cost of inhibition on the presynaptic one. Flow occurs in a balancing way in order to even out deviations from an equilibrium state (Bennett & Zukin, 2004; Connors & Long, 2004; Hormuzdi *et al.*, 2004). Thus the definition of pre- and post- synaptic can be ambiguous when considering an electrical synapse as it will depend on a bias introduced by the observer rather than a true directionality of the signal. If one is considering excitatory events then will consider the postsynaptic cell as being post-synaptic but given that as this cell becomes depolarized the other becomes hyperpolarized it could also be considered pre-synaptic to an inhibitory event. Gap junctions act as low pass filters and as a consequence the action potential itself has generally little impact across an electrical synapse which highly contrasts with chemical transmission for which it is the ultimate trigger (Bennett & Zukin, 2004; Connors & Long, 2004; Hormuzdi *et al.*, 2004). Slow subthreshold fluctuations in membrane potential are the optimally transferred stimuli while faster stimuli such as action potentials are filtered. The temporal aspect of transmission also contrasts highly, while chemical transmission is rapidly terminated either by removal/degradation of the neurotransmitter molecule and/or desensitization of the postsynaptic receptors there are no time constraints (apart from the filtering of very fast events) on stimuli flowing through gap junctions which can last several milliseconds. These are features that make electrical synapses very efficient in synchronizing ensembles of interconnected cells and considered to be one of the main advantages of gap junctions (Bennett & Zukin, 2004; Connors & Long, 2004; Hormuzdi *et al.*, 2004).

In several brain regions in which such subthreshold oscillations are thought to play a crucial role it has been observed that ensembles of (electrically) interconnected

cells tend to be functionally similar and in close spatial proximity (Galarreta & Hestrin, 1999; Gibson *et al.*, 1999; Galarreta *et al.*, 2004; Hestrin & Galarreta, 2005). While often the emergence of such oscillations does not require gap junctions and probably arise due to precisely timed inhibitory and/or excitatory inputs, gap junctions have been shown to dramatically increase the synchrony of such oscillations across the interconnected cells (Bennett & Zukin, 2004; Connors & Long, 2004; Hormuzdi *et al.*, 2004). Among these structures are the inferior olivary nucleus (Long *et al.*, 2002), thalamic reticular nucleus, hippocampus (Hormuzdi *et al.*, 2001; Buhl *et al.*, 2003) and neocortex where they interconnect homogenous populations of interneurons. Gap junctions are also abundant in the retina, which is one of the few structures in which it has been shown that between heterologous cells and when input resistances differ largely, unidirectional gap junction signaling can occur (Veruki & Hartveit, 2002). In the olfactory bulb it has been shown that gap junctions interconnect granule cells (Reyher *et al.*, 1991) and that mitral cells sharing the same glomerulus are connected, whereas coupling between non-glomerular partner never occurs. This has been proposed to coordinate subthreshold oscillations (Friedman & Strowbridge, 2003) and spike synchrony (Schoppa & Westbrook, 2001, 2002; Migliore *et al.*, 2005) between mitral cells.

Chemical transmission in the nervous system

Chemical synapses as mentioned earlier rely on a completely different mechanism for the transmission of information between neurons. At these synapses there is no continuity between the cytoplasm of the presynaptic and postsynaptic cells. Instead the presynaptic neuron releases small molecules, neurotransmitters, into the small space between the two membranes, the synaptic cleft. Neurotransmitter molecules diffuse in the extracellular space and activate receptors in the postsynaptic cell. These receptors transduce the binding of the neurotransmitter molecules into an electrical or a modulatory signal. This type of transmission imposes temporal constraints to the stimulus. Unlike for electrical synapses in which the stimulus is

readily transmitted, in chemical synapses there is an imposed latency of a few milliseconds (Sabatini & Regehr, 1999) to which a rapid termination of the stimulus is enforced by the removal/degradation of the neurotransmitter or the inactivation of its receptors (Jones & Westbrook, 1996). Information flows asymmetrically, unlike in electrical synapses, from one clearly identifiable presynaptic cell to a postsynaptic cell. The specificity and precision of the direction of information and the fact that all the steps from the release of neurotransmitter from the presynaptic terminal to the transduction in the postsynaptic cell can be regulated has placed chemical synapses at the center of the search for the neural correlates that underlie higher cognitive functions such as memory formation and retrieval (Malenka & Bear, 2004). Chemical transmission relies on a vast repertoire of transmitters and receptor molecules. The most abundant neurotransmitters are, as will be discussed later, glutamate (excitatory) (Ozawa *et al.*, 1998) and GABA (inhibitory) (Macdonald & Olsen, 1994). In order to translate signals by neurotransmitters neurons rely on an even larger repertoire of receptor molecules. This allows the same neurotransmitter to induce distinct responses depending on the type of receptors that are expressed. In general receptors, regardless of which neurotransmitter they respond to, can be subdivided into two main categories: ionotropic and metabotropic receptors (Ozawa *et al.*, 1998).

Ionotropic receptors are those that operate via the opening of a channel, gated by the neurotransmitter, which is usually selective to a given ion/s. The receptor's channel is normally closed and opens for a brief duration as a consequence of the binding of neurotransmitter molecules. This allows for influx or efflux of permeable ions which will directly cause either a depolarization or hyperpolarization of membrane. Thus ionotropic receptors directly influence the membrane potential of the cell (Ozawa *et al.*, 1998).

In contrast metabotropic receptors when activated initiate an intracellular signaling cascade which usually modulates the neurons excitability by the activation of

enzymes such as kinases or phosphatases. This could lead to several consequences for synaptic transmission, such as, increasing/decreasing ionotropic receptor number, conductivity, open probability, increase/decrease of release probability via modulation of Ca^{2+} channels. Alternatively it can directly modulate the membrane excitabilitity, for instance by activating/inactivating voltage sensitive K^+, Na^+ or Ca^{2+} channels. Metabotropic receptors by tampering with the cells excitability generally operate by influencing the integration of inputs rather than providing a direct depolarizing or hyperpolarizing signal (Ozawa *et al.*, 1998).

Glutamate and its receptors

Fast excitatory transmission in the central nervous system (CNS) is largely mediated by glutamate and its ionotropic receptors. The latter can be subdivided into two main classes, according to characterization made with the use of exogenous agonists, the NMDA (n-methyl-D-aspartate) receptors and the non-NMDA receptor which include the two subfamilies AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) and kainate receptors. The last group (non-NMDA) is largely responsible for the fast component of excitatory transmission which is mainly due to Na^+ influx as these receptors have in general very low conductance to Ca^{2+}. Kainate receptors desensitize very rapidly leaving the AMPA receptors responsible for the bulk component of rapid postsynaptic currents. NMDA receptors contrast with AMPA/kainate receptors firstly in being highly permeable to Ca^{2+} and secondly by being voltage dependent in their activation (Mayer, 2005). Due to an Mg^{2+} block these receptors have no conductance at resting membrane potential whereas at more depolarized potentials this block is relieved allowing cation influx upon opening. Considering that most glutamatergic synapses express both kinds of receptors, the depolarization required to remove the Mg^{2+} block could come from AMPA mediated currents suggesting that the NMDA receptors could function as coincidence detectors for pre and post-synaptic activity providing a Ca^{2+} trigger for

intracellular cascade dependent processes or even neurotransmitter release (Malenka & Bear, 2004).

While glutamate is the main excitatory neurotransmitter, GABA (γ-amino-butyrate) is responsible for most of the ionotropic inhibitory transmission in the CNS. GABA functions mainly via the $GABA_A$ receptor which once activated allows the influx of Cl^- ions leading to membrane hyperpolarization (Macdonald & Olsen, 1994). Alternatively GABA can also activate $GABA_B$ receptors which initiate an intracellular cascade (metabotropic) which normally leads to the inactivation of Ca^{2+} channels decreasing the release probability or the activation of K^+ channels which decreases the overall excitability of the cell. Among other neurotransmitters are acetylcholine, glycine, dopamine, ATP and serotonin, though for the purpose of this thesis glutamate and its ionotropic receptors are the most relevant to describe in detail.

The diversity of AMPA receptors

AMPA receptors, as mentioned before, fall into the ionotropic class of receptors. They are composed by the assembly of 4 subunits from a range for 4 different subunits (GluR1-4) each subunit has its own splice variants providing several possible combinations of functional receptors. They are often heteromeric though it is possible to form functional homomeric channels (Ozawa *et al.*, 1998). Different subunit composition confers different properties to these receptors such as open probability, channel conductance and even ion selectivity. Most AMPA receptors are selective for monovalent cations. Due to post-transcriptional editing of the GluR2 subunit the change of a single amino-acid residue in a pore motif is enough to render receptors containing this subunit effectively Ca^{2+} impermeable. Receptors lacking GluR2 subunit or assembled with an unedited (less frequent) version of it have distinct properties when compared to those incorporating the edited version (Cull-Candy *et al.*, 2006). One of the most striking differences and possibly the most functionally relevant is the difference in Ca^{2+} permeability. Due to the contrast with

the most common version of AMPA receptors which are impermeable to this ion, GluR2 lacking receptors are known as calcium permeable AMPA receptors (CP-AMPA receptors; Cull-Candy *et al.*, 2006). Another contrasting feature is that these receptors have a non-linear I/V relationship. They display inward rectifying properties due to a block by endogenous polyamines to which calcium impermeable AMPA receptors are insensitive. CP-AMPA receptors have been implicated in several forms of plasticity in various brain regions such as the hippocampus and amygdala (Isaac *et al.*, 2007).

Dendrites as transmitters

Conventional synaptic transmission occurs due to release of neurotransmitter from axonic terminals to activate a postsynaptic specialization in the dendrites of other neurons (although axons can also be targets) or target structures such as a muscle fiber. Thus dendrites are classically viewed as the integrators of the various synaptic signals which are received in various locations throughout its dendritic arbor at a given time. Several factors dictate whether or not a cell discharges one (or more) action potential and among these are the relative timing and locations along the dendrite of the inputs along with the passive and active properties of the dendritic membrane.

In addition information processing in various brain structures such as the substantia nigra (Bjorklund & Lindvall, 1975; Drake *et al.*, 1994; Hausser *et al.*, 1995), hippocampus (Drake *et al.*, 1994; Wilson & Nicoll, 2002), cerebellum (Glitsch et al., 1996) and the cortex (Zilberter et al., 1999) involves the release of neuromodulators and/or transmitters from neuronal dendrites. Studies indicate transmission from dendrites is typically initiated by local axonal input, requires postsynaptic depolarization and acts largely to provide a modulatory feedback signal (Ludwig & Pittman, 2003). In the olfactory bulb, however it is the release of glutamate from mitral and tufted cell dendrites onto local inhibitory interneurons which provides

recurrent and lateral signaling throughout the olfactory bulb circuitry (Pinching & Powell, 1971a; Jahr & Nicoll, 1980; Aungst *et al.*, 2003; Murphy *et al.*, 2005). Thus much like an axon, dendrites can mediate the spread of information as well as receiving and integrating it (for an overview; Margrie & Urban, 2007). This is certainly the case in the olfactory bulb in which most of the synaptic transmission occurs via dendro-dendritic synapses. Mitral cells are known to have synaptic vesicles loaded with glutamate located all throughout their lateral dendrites and apical tuft. On the other hand the axonless granule cells contain GABA as a neurotransmitter on their dendrites forming dendrodendritic reciprocal synapses with mitral cells (Rall *et al.*, 1966; Isaacson & Strowbridge, 1998; Aroniadou-Anderjaska *et al.*, 1999).

It is thought that in most cases dendritic release follows most characteristics of axonal release although it is not clear if the machinery is exactly the same. Influx of calcium is a requirement for release which in most cases is thought to arise directly from backpropagating action potentials (Isaacson & Strowbridge, 1998; Isaacson, 1999). In mitral cells action potentials can fully backpropagate into the apical tuft thus providing the depolarization necessary for reliable release of neurotransmitter (Bischofberger & Jonas, 1997; Chen *et al.*, 1997). In the lateral dendrites it is thought that the extent of back-propagation of action potentials is regulated by several factors such as activation of potassium channels, number of APs and inhibitory events (Margrie *et al.*, 2001; Lowe, 2002; Christie & Westbrook, 2003; Halabisky & Strowbridge, 2003). Therefore the reliability of release at a given dendritic location is naturally also dependent on these factors which will be determinant in the regulation of lateral inhibition (Urban, 2002; Urban & Sakmann, 2002; Schoppa & Urban, 2003; Egger & Urban, 2006), a phenomenon which has been shown to be crucial in olfactory discrimination (Shimshek *et al.*, 2005). Thus dendrites are not constrained to receiving and integrating input but in some brain structures, like the olfactory bulb, they are in fact the main transmitters.

Statistical nature of chemical transmission

Release occurs in a probabilistic fashion. Pioneering work by Katz and colleagues gave birth to the first model attempting to explain the rules of transmitter release, the quantal model (Del Castillo & Katz, 1954b). Such a model aimed to describe neurotransmission at the neuromuscular junction which despite the anatomical differences to central synapses, has been successfully adapted to explain the properties of central synaptic transmission (Isaacson & Walmsley, 1995; Borst & Sakmann, 1996; Silver *et al.*, 1998). Analysis of the end plate potential in the muscle fiber revealed that spontaneously occurring events varied little in amplitude and corresponded to the smallest measurable postsynaptic response (often referred to as miniature potentials or minis; Del Castillo & Katz, 1954b). Thus such responses were treated as quantal entities and predicted that the observed evoked responses could be explained as the result of linear summation of several quanta and thus the response corresponds to a multiple of the quantal response (Del Castillo & Katz, 1954b).

The quantal model assumes that each synapse has a number of release sites (N) that operate independently. Either transmission fails or a single vesicle is released. The reliability of the synapse is reflected in the release probability (P_r). This has been shown to be dependent on several factors and that different synapses operate with different release probabilities even when belonging to the same axon terminal or when sharing the same post-synaptic partner (reviewed in Walmsley *et al.*, 1998). Thus the presynaptic element will contribute for the communication between two given neurons with a given number (N) of release sites which express a given probability of releasing (Pr) neurotransmitter upon an action potential; N x Pr (Redman, 1990).

The amplitude of the response in the post synaptic cell will be dependent on the quantal size which is the postsynaptic response to the release of a single quantum. This is in turn also dependent on several factors such as the number of receptors and their biophysical properties such as their open probability and the conductance of each single channel. Given these parameters we can describe the mean post-synaptic response to a presynaptic action potential as the product of the presynaptic contribution and the quantal size (Korn & Faber, 1991).

The probabilistic nature of chemical transmission reflects another fundamental difference with electrical synapses. Due to this, chemical synapses are somewhat less reliable than electrical ones. Gap junction mediated transmission faithfully follows the temporal kinetics of the presynaptic stimulus (except perhaps for very fast events such as action potentials) and its response is proportional to the presynaptic activity, that is the amount of depolarization is relevant to the transmitted signal (though it does not determine it exclusively). In contrast chemical transmission is an all or none event, it does not depend proportionally on the amount of depolarization experienced by the presynaptic cell provided an action potential is triggered. Given the occurrence of action potential discharge then transmission at a given synapse might or might not occur depending on the factors mentioned above.

The short-term dynamics of chemical synapses

The fact that most of the parameters (N, Pr, q) mentioned above can be independently modulated results in chemical synapses not being rigid transmitting structures but rather expressing use dependent dynamic changes in transmission at various time scales (Zucker & Regehr, 2002). Several forms of changes in synaptic efficacy have been reported and while some exhibit short term durations other can last several hours/days. The recent (less than a minute) history of activation has been shown to strongly influence the efficacy of chemical transmission (Del Castillo

& Katz, 1954a). A common demonstration of such phenomena comes from paired pulse stimulation experiments. In such assays two events are evoked with a short interstimulus interval, in such conditions usually either an enhancement or depression of the second event when compared to the first is observed.

The use dependent short term increase in synaptic efficacy is often referred to as facilitation and it is generally accepted that presynaptic calcium plays a major role in the mechanism of this type of short term of plasticity (Kamiya & Zucker, 1994). When the soma of a neuron becomes depolarized enough to reach action potential threshold, the generated action potential propagates along the axon and into the synaptic boutons. Depolarization of the bouton activates voltage sensitive calcium channels leading to a rise in intracellular calcium levels which is later buffered by calcium homeostasis mechanisms. The rise in Ca^{2+} concentration triggers vesicular release, by binding to the fusion regulatory proteins, such as synaptotagmin. This induces vesicular fusion allowing for the packed neurotransmitter to be released onto the synaptic cleft. Release probability is tightly dependent on calcium levels and it has been shown that removing extracellular calcium effectively shuts down transmission while increasing calcium level enhances the release of neurotransmitter. It is thought that, should a second event (AP) occur in a time frame that is not sufficient for calcium homeostasis to restore basal calcium concentrations, then the influx of calcium via voltage sensitive calcium channels builds up on the residual leftover from the previous event to cooperatively bind to calcium sensors resulting in a temporary increase in the release probability (Zucker, 1973). Such a transient increase in release probability results in an enhanced or facilitated post-synaptic event. The residual calcium hypothesis (Kamiya & Zucker, 1994) has been widely accepted but nevertheless other factors can contribute to facilitation. Examples include synapses that incorporate calcium permeable AMPA receptors that can show facilitation due to a use-dependent relief of polyamine block (Isaac *et al.*, 2007).

At different synapses however repetitive stimulation leads to a short-term depression of the postsynaptic response under the same experimental conditions (such as extracellular calcium levels and temperature; (Reyes *et al.*, 1998; Zucker & Regehr, 2002). Like for facilitation it is thought that the mechanism, although being different, is mainly pre-synaptic in origin. In situations of high release probability temporary depletion of the readily releasable pool of synaptic vesicle can occur at the release sites which results in the depression in the post-synaptic response despite the increase in calcium concentration in the terminal mentioned before (Zucker & Regehr, 2002). Post synaptic mechanisms can also contribute to short-term depression. The biophysical properties of receptors and even the geometry of the synaptic cleft can influence the way repeated use of a synapse is read out on the target cell (Walmsley *et al.*, 1998). Receptor open probability depends on neurotransmitter concentration. In a scenario of high frequency stimulation it is likely that neurotransmitter concentration is increased. However if the postsynaptic receptors are fully occupied/open the synapse would fail to display a facilitated response (Foster *et al.*, 2002). In addition desensitization of post-synaptic receptors has been shown to lead to depression of postsynaptic responses (Jones & Westbrook, 1996; Ozawa *et al.*, 1998).

Short term facilitation or depression are not mutually exclusive to a given axon. In necortical circuits it has been shown that for the same presynaptic cell synapses to different targets express different short-term dynamics (Reyes *et al.*, 1998; Rozov *et al.*, 2001). Short-term dynamics are thought to reflect the functional signature of the synapse and in accordance across different cell types and regions in the brain different ranges of depression or facilitation are observed. Although short-term dynamics in general are thought to be mainly determined by the pre-synaptic mechanisms, as mentioned above several post synaptic properties also contribute. Nevertheless it is expected that changes in release probability should lead to profound changes in the short term dynamics at a given synapse (Silver *et al.*, 1998). Changes in the short-term dynamics of a synapse are often used as

indicators for site of expression in long term changes in synaptic efficacy (Korn & Faber, 1991).

Long-term changes in synaptic efficacy

Under particular stimulation conditions, synapses are known to undergo a dramatic increase in synaptic efficacy that can last for hours, days or weeks a phenomenon called long–term potentiation (LTP). This phenomena has most exhaustively been studied in the hippocampus where it was first observed (Lomo, 1968) but since then been observed at many synapses in the nervous system (reviewed in Citri & Malenka, 2008). The cellular mechanisms have been intensively studied over the past years and it is generally accepted that there are two main distinct mechanisms of induction. One is dependent on the synaptic activation of the NMDA receptors and another that does not require the activation of these receptors (Citri & Malenka, 2008).

At the CA1 region of the hippocampus upon strong synaptic activation the postsynaptic cell depolarizes sufficiently to relieve the Mg^{2+} block on the NMDA channels allowing the influx of Ca^{2+} (Malenka, 1991). The increase in the intracellular concentration of this ion activates the kinase CaMKII (calcium/calmodulin dependent kinase II; Barria *et al.*, 1997) which once active can phosphorylate a number of substrates and activate intracellular signaling cascades such as the mitogen-activated protein kinase (MAPK), phophoinositide 3-kinase (PI3K) and the protein kinase C (PKC). In an early phase the changes in synaptic efficacy are thought to be dependent mainly on the increase in the number of AMPA receptors (Hayashi *et al.*, 2000; Malinow *et al.*, 2000) or the change of their biophysical properties by direct phosphorylation. Thus the locus for the observed change lies in modifications to the postsynaptic cell (Malenka & Bear, 2004).

A longer lasting phase, the so called late phase, of LTP was shown to be dependent on transcription and synthesis of new proteins and even morphological changes in dendritic spines (Kandel, 2001; Bailey & Kandel, 2008). The initiation of intracellular signaling pathways leads to the activation of activity-dependent transcription factors or modulators which leads to the upregulation of a number of genes called immediate early genes which are thought to be required for the maintenance of the LTP. Not all of these genes/proteins have been identified nor their precise roles in the maintenance of LTP. Among these are glutamate receptor subunits, transcription factors such as c-fos and cytoskeleton proteins such as Arc (activity regulated cytoskeleton protein; Citri & Malenka, 2008) .

In contrast to LTP at CA1 synapses, at the mossy fiber to CA3 synapse LTP is not dependent on the activation of NMDA receptors and has a presynaptic expression site (Zalutsky & Nicoll, 1990). However the induction of changes in efficacy also strictly depends on the increase of Ca^{2+} intracellular levels. This leads to an increase in cyclic AMP (cAMP) which by activating intracellular signaling pathways will culminate in changes in the exocytotic apparatus resulting in increased transmitter release. The maintenance of this form of LTP has been shown to depend on new protein synthesis but does not require transcription (Citri & Malenka, 2008).

As with short term dynamics, synaptic transmission can also undergo long-term depression (LTD). Once more one of the key mediators is Ca^{2+} influx through NMDA receptors. LTD is generally induced by stimulation in lower frequencies than those required for LTP induction and therefore leading to an intermediate Ca^{2+} increase when compared to that observed in LTP (Dudek & Bear, 1992). Therefore the levels of calcium increase have been proposed to be the determinant for the balance/switch between triggering LTP, LTD or no change in synapse efficacy. LTD induction is also dependent on signaling pathways but this time leading mainly to an activation of phosphatases instead of kinases. Targets for these phosphatases are

once more the AMPA receptors which can be very rapidly internalized. There are also NMDA independent mechanisms to induce LTD which require the activation of metabotropic glutamate receptors which operate by a pre-synaptic mechanism (Citri & Malenka, 2008). For long there has been a debate on where is the site of expression of long term plasticity, nowadays it is believed that both pre and post mechanisms can play a role and that most likely structural changes which lead to the formation/activation of new synapses are involved in the longer term stabilization of the plastic change (Edwards, 1995; Nagerl *et al.*, 2004; Holtmaat *et al.*, 2006).

As briefly suggested above, one indicator that is often used to provide a clue on the locus for plasticity are changes in the short-term dynamics of the synapse. Such a change is thought to reflect modifications in the release probability and thus if it is accompanied by a change in synaptic efficacy it is thought to support the case for a presynaptic locus of expression. Quantal analysis has also been used to dissect the locus of synaptic efficacy changes however it requires quantification of all the quantal factors which is often not possible due to experimental constraints. In situations where the quantal content cannot be resolved, indirect methods have been implemented such as coefficient of variation (CV) analysis (Faber & Korn, 1991). The CV can be calculated according the following equation: $CV = \sigma/M$ in which σ is the standard deviation of the response and M is the mean synaptic response. The method predicts that CV is only dependent on the presynaptic parameters. Thus the rationale is that a purely post-synaptic change should not result in changes in the coefficient of variation and in a purely presynaptic locus it should change in a way that follows the ratiometric change in amplitude (Korn & Faber, 1991).

The relevance of the various types of stimuli used for plasticity induction has also received much attention (Paulsen & Sejnowski, 2000). The first observations were made with the use of very long high frequency stimulation (Bliss & Lomo, 1973)

which is thought unlikely to occur *in vivo* and inconsistent with observations in which the data rather suggested that activity in the hippocampus was dominated by slow frequency waves within the theta range (4-7 Hz) and thus spiking activity should follow such oscillations (Paulsen & Sejnowski, 2000). The search for more physiological patterns of stimulation has led to adapting the induction protocol with a pattern that follows the theta rhythm (theta burst stimulation - TBS; Larson *et al.*, 1986). The stimulus was constructed using short bursts of high frequency stimuli which were interspaced to match the range of theta frequency (~5Hz). This pattern of stimulation proved to be more effective in inducing potentiation at the CA1 region of the hippocampus (Larson *et al.*, 1986). It is now understood that neuronal activity in these regions is indeed tightly linked to the occurring theta rhythms and thus stimuli that follow such patterning are more likely to mimic physiological stimuli (Paulsen & Sejnowski, 2000). Since then it has been found TBS was also successful at inducing plasticity in other brain regions such as the piriform cortex. More refined protocols have been tested since, in particular those that allow a tighter control of the timing of pre-synaptic and post-synaptic action potentials. In such experiments much milder and physiological induction protocols in which the pre-synaptic action potential is carefully timed to precede or succeed the post-synaptic backpropagating action potential has led to the finding that (for instance in the cortex) there is a considerably narrow time window for the coupling of presynaptic and postsynaptic activity (Markram *et al.*, 1997; Sjostrom *et al.*, 2001). More remarkably, depending on which preceded the other, the outcome could be either depression or potentiation, a phenomena that has been called spike-timing dependent plasticity (STDP; Markram *et al.*, 1997; Sjostrom *et al.*, 2001; Sjostrom & Hausser, 2006).

Changes in synaptic strength aren't the only mechanisms by which a neuronal network can change its own efficacy. Altering the neuron's intrinsic excitability can have a dramatic effect on network dynamics and might even be a requirement for the maintenance of network homeostasis and stability. Such changes are not as

thoroughly studied as synaptic transmission plasticity but have been reported to occur in an activity dependent manner and in some cases to be coupled with LTP (Turrigiano, 2007). These mechanisms include changes in action potential threshold, in input resistance and also changes in the distribution and properties of channels such as Na^+ and K^+ voltage sensitive channels in the cellular membrane. All such factors can have an effective impact on the input/output ratios potentially necessary for sensory processing and other higher order functions (Turrigiano, 2007).

Anatomical and synaptic organization of the mammalian olfactory bulb

The olfactory bulb is the first processing structure in the olfactory system. The olfactory signal originated in the olfactory sensory neurons is directly transmitted to cells within this structure which will then relay it to various other structures including the piriform cortex, amygdala and enthorinal cortex (Wilson & Mainen, 2006). In structures such as the olfactory cortex, integration and association of this sensory modality with other modalities occurs to ultimately compute a behavioural response.

In the nasal epithelium the olfactory receptor neurons (ORNs) transduce odor stimuli into an electrical signal. This is initiated by the binding of airborne odor molecules onto the binding site of olfactory receptors (ORs). These receptors are part of a large multigene family of G protein coupled receptors (Buck & Axel, 1991). Therefore the binding of such molecules triggers an intracellular signaling cascade that will lead to the depolarization of the neuron and to the triggering of action potentials. This occurs via an increase in cAMP which opens a nucleotide gated channel (CNG) allowing the influx of calcium which will ultimately result in the release of glutamate onto the downstream cells in the olfactory bulb (Bhandawat *et al.*, 2005). In order to faithfully represent the olfactory external world the mouse relies on a repertoire of 1000 different receptors whose main difference is the fact that their affinities to the various odour molecules varies substantially (Fuss &

Korsching, 2001). Each of these receptor molecules can be activated by a range of olfactory molecules, rather than specifically binding a single odour molecule the receptors are selective to a given molecular feature (such as a functional group or a side chain) enabling each receptor to be activated by a range of structurally similar odorants, though with different affinities (Duchamp-Viret *et al.*, 2000; Fuss & Korsching, 2001). Likewise the same odour molecule has been shown to activate more than one receptor molecule. For these reasons olfactory sensory neurons are functionally segregated and restrained to the encoding of a small fraction of the spectrum of olfactory stimuli (Duchamp-Viret *et al.*, 2000).

Those ORNs expressing the same receptor converge their axons onto the same discrete structure on the surface of the main olfactory bulb (MOB), termed the glomerulus (Buck & Axel, 1991; Mombaerts *et al.*, 1996). The glomeruli are constituted by the neuropil of the sensory neurons axon terminals and the apical dendritic tufts of downstream cells: the mitral cells and the juxtaglomerular cells. These structures have sphere-like shapes which are surrounded by glia cells and the somas of the heterogenous group of neurons named juxtaglomerular cells (JC) (Kosaka & Kosaka, 2005a). The glomerular layer constitutes the most superficial cellular layer of the olfactory bulb which is surrounded by the plexiform layer, olfactory nerve layer (ON) which contains the axon terminals of olfactory sensory neurons (Figure 1.1).

Such anatomical segregation of functionally identical sensory afferents into the olfactory bulb leads to the consequence that a given odor stimulus will lead to the activation of a unique set of glomeruli (figure 1.1; Wachowiak & Cohen, 2001; Bozza *et al.*, 2002). Therefore such a functional and anatomical arrangement should lead to the emergence of a spatial map of glomerular activity in the olfactory bulb which should translate as incoming excitatory sensory drive into each individual glomerular network. The emergence of such a spatial map has been confirmed by several lines of evidence (Bozza & Mombaerts, 2001; Luo & Katz,

2001; Meister & Bonhoeffer, 2001; Wachowiak & Cohen, 2001; Bozza *et al.*, 2004). In addition it was found that there is a stereotyped anatomical glomerular map in the mouse, which has led to the molecular/functional identification of some glomeruli (Meister & Bonhoeffer, 2001; Johnson & Leon, 2007). This has enabled better understanding of the receptive range of individual glomeruli. The formation of such spatial map of activity in the olfactory bulb is thought to underlie odor representation at this early stage of processing and the activity of each glomerulus is thought to reflect a given molecular feature of an odor stimulus at the level of the olfactory bulb (Belluscio *et al.*, 2002; Bozza *et al.*, 2002).

In the mouse olfactory bulb, cells that receive direct sensory input commit to doing so exclusively via a single glomerulus. Mitral cells have a single apical dendrite that projects into a glomerulus as do juxtaglomerular cells. Therefore the glomerulus further functionally defines a subnetwork of cells which commit to receiving similar sensory input (Koulakov *et al.*, 2007). Despite a wealth of knowledge concerning the molecular and anatomical organization of sensory input to glomeruli, our understanding of how neuronal networks within this structure integrate sensory activity remains unknown. Each glomerulus functionally circumscribes columns of about 25-50 mitral/tufted cells (MCs) which are the olfactory bulb principal cells. They receive direct glutamatergic input from the ORNs and are responsible for transmitting the locally refined signal onto the olfactory cortex and other regions of the brain downstream of the olfactory bulb . However sensory neurons do not synapse exclusively onto mitral cells but also to local interneurons (i.e. juxtaglomerular cells) and several lines of evidence suggest that the representation of odours in the olfactory bulb is more complex than the formation of a hardwired spatial map and that such representations are temporally structured (Spors & Grinvald, 2002; Broome *et al.*, 2006; Schaefer & Margrie, 2007). In order to understand the processing taking place within the olfactory bulb it is crucial to understand how its building blocks dynamically interact with each other as well as understanding how cells within the same glomerular module contribute to

glomerular output. In the mouse olfactory bulb interactions between different glomerular modules are thought to be predominantly inhibitory. These are normally multisynaptic interactions which can be mediated via granule cells, which directly inhibit mitral cells (Isaacson & Strowbridge, 1998) or a subgroup of juxtaglomerular cells, the short axon cell which activates inhibitory periglomerular neurons which then impact on mitral cell activity (Aungst *et al.*, 2003; Hayar *et al.*, 2004). It has been proposed that these inhibitory interactions are crucial in the shaping olfactory bulb activity and increasing the contrast between active glomeruli (Urban, 2002). Interactions between glomerular modules can occur at the level of the glomerular layer or at the level of the granule cell layer. Apart from mitral/tufted cells which are the olfactory bulb output cells most cells that synapse within the olfactory bulb are axonless. The exceptions are a subgroup of juxtaglomerular cells called short axon cells and the external tufted cells. These two types of cells are glutamatergic. Short axon cells receive excitatory input from the external tufted cells and synapse onto periglomerular cells that belong to a different glomerulus (Aungst *et al.*, 2003). External tufted cells are driven by direct sensory input and in addition to exciting short axon cells also synapse onto periglomerular cells (Hayar *et al.*, 2004). Periglomerular cells can be GABAergic or dopaminergic and directly inhibit both mitral cells, with which they form dendrodendritric synapses, and the axon terminals of sensory neurons (Parrish-Aungst *et al.*, 2007). Although short axon cells are glutamatergic, together with periglomerular cells they contribute to lateral inhibition of cells that belong to another glomerular module.

Models of olfactory bulb function

It as been proposed that the synaptic organization of the olfactory bulb in which functional modules interact with others via inhibitory lateral interactions operates as a contrast enhancement device (Margrie & Schaefer, 2003; Schoppa & Urban, 2003; Arevian *et al.*, 2008). Indeed in vivo manipulations to lateral and recurrent inhibition has been shown to have profound effects on the ability of mice to perform

in an odour discrimination task (Shimshek *et al.*, 2005). The implications of such an arrangement could simply be to refine a spatial map which would later be translated into an odor identity. However it is clear that odor representation in the olfactory bulb is temporally structured and representation as a static map does not accommodate the observations that have been made from populations of olfactory bulb cells. Furthermore it is unlikely to account for all the necessary computation that is required from the olfactory system such as background subtraction (Brody & Hopfield, 2003; Scott *et al.*, 2006; Schaefer & Margrie, 2007).

It is also clear that the olfactory bulb network is dominated by subthreshold oscillations (Carlson *et al.*, 2000; Cang & Isaacson, 2003; Margrie & Schaefer, 2003). Mitral cells *in vivo* show rhythmic fluctuations in membrane potential in a frequency and phase that match that of the respiratory cycle (theta rhythm) which is a consequence of the active sampling of odours (sniffing; Cang & Isaacson, 2003; Margrie & Schaefer, 2003; Schaefer & Margrie, 2007). The olfactory bulb network has also been observed to express faster oscillations (gamma range 20-70 Hz) which is thought to be a direct consequence of the intrabulbar circuitry (Friedman & Strowbridge, 2003; Halabisky & Strowbridge, 2003; Galan *et al.*, 2006). These have been proposed to play a crucial role in olfactory processing increasing the computational power of the network by the means of increasing spike precision in mitral cells (Balu *et al.*, 2004; Schaefer *et al.*, 2006). This would support a latency onset based olfactory code which would rely on a high precision of the onset of mitral cell responses combined with the spatial location pattern of activation (Schaefer & Margrie, 2007). The respiration induced oscillation forces mitral cell activity to also follow this rhythm. Mitral cells tend to fire a few action potentials in each sniff cycle at a fixed frequency (Cang & Isaacson, 2003; Margrie & Schaefer, 2003). Models that incorporate network oscillations as having an active role in processing usually come hand in hand with synchrony between functionally similar mitral cells in playing an equally important role (Cleland & Linster, 2002; Brody & Hopfield, 2003; Balu *et al.*, 2004). Several behavioural studies have shown that

mice can discriminate between odour within one sniff which implies that each sniff cycle and thus oscillatory cycle carries odor specific information (Uchida & Mainen, 2003; Abraham *et al.*, 2004; Kepecs *et al.*, 2006; Rinberg *et al.*, 2006; Verhagen *et al.*, 2007). It is thought that the ensemble of intraglomerular mitral cells acts as a whole to carry highly synchronous input into downstream target structures (Suzuki & Bekkers, 2006). In the slice mitral cells show a remarkable synchrony both in spontaneous subthreshold depolarization and action potential discharge (Carlson *et al.*, 2000; Schoppa & Westbrook, 2001, 2002).

Among the mechanisms implicated in such synchrony are dendritic auto-receptors and gap junction mediated coupling (Schoppa & Westbrook, 2001, 2002; Christie *et al.*, 2005). Mitral cell dendrites are known to contain glutamatergic vesicles both in their apical and lateral dendrites that mediate recurrent and lateral inhibition via local interneurons. Though MCs are known to form dendrodentritic synapses with periglomerular cells within the glomerulus they are thought not to form synaptic contacts with other mitral cells (Pinching & Powell, 1971c, b, a, 1972; Kosaka & Kosaka, 2005b). Release of glutamate from mitral cell dendrites has been shown to evoke both AMPA and NMDA receptor-mediated potentials in the same MC which is referred to as self-excitation (SE) (Isaacson, 1999; Margrie *et al.*, 2001; Salin *et al.*, 2001; Schoppa & Westbrook, 2001, 2002). Gap junctions located in the tuft, proximal to the source of self excitation (Salin *et al.*, 2001) are thought to electrically transmit EPSP-like depolarisations through the mitral cell network (Schoppa & Westbrook, 2002; Christie *et al.*, 2005; Christie & Westbrook, 2006). Such phenomena have been named lateral excitation and shown to result in the glomerulus specific synchronization of the mitral cell ensemble (Schoppa & Westbrook, 2001, 2002; Christie *et al.*, 2005; Christie & Westbrook, 2006).

This work aimed to investigate the modes of communication that are available to mitral cells sharing the same intraglomerular network and to explore directly the relationship between chemical and electrical transmission in intraglomerular mitral

cell networks. In addition plasticity within the olfactory bulb dendro-dendritic network has long been suggested to provide a substrate for olfactory memory, (Rosser & Keverne, 1985; Kaba & Keverne, 1988; Sullivan *et al.*, 2000) sensory adaptation (Li, 1990), tuning receptive fields and even discrimination learning (Wilson *et al.*, 2004). However direct evidence to support such kind of neuronal plasticity between dendritic connections in the olfactory bulb or even for dendritic transmission in general has not materialized. This raises the question of whether dendritic transmission is functionally more hard-wired than conventional axonal transmission and whether dendro-dendritic transmission in the olfactory bulb (or in general) can provide the kind of activity dependent plasticity observed at many axo-dendritic synapses. I therefore also examined the possibility that the lateral excitatory connection between mitral cells might provide the substrate for long term changes in synaptic efficacy in the olfactory bulb.

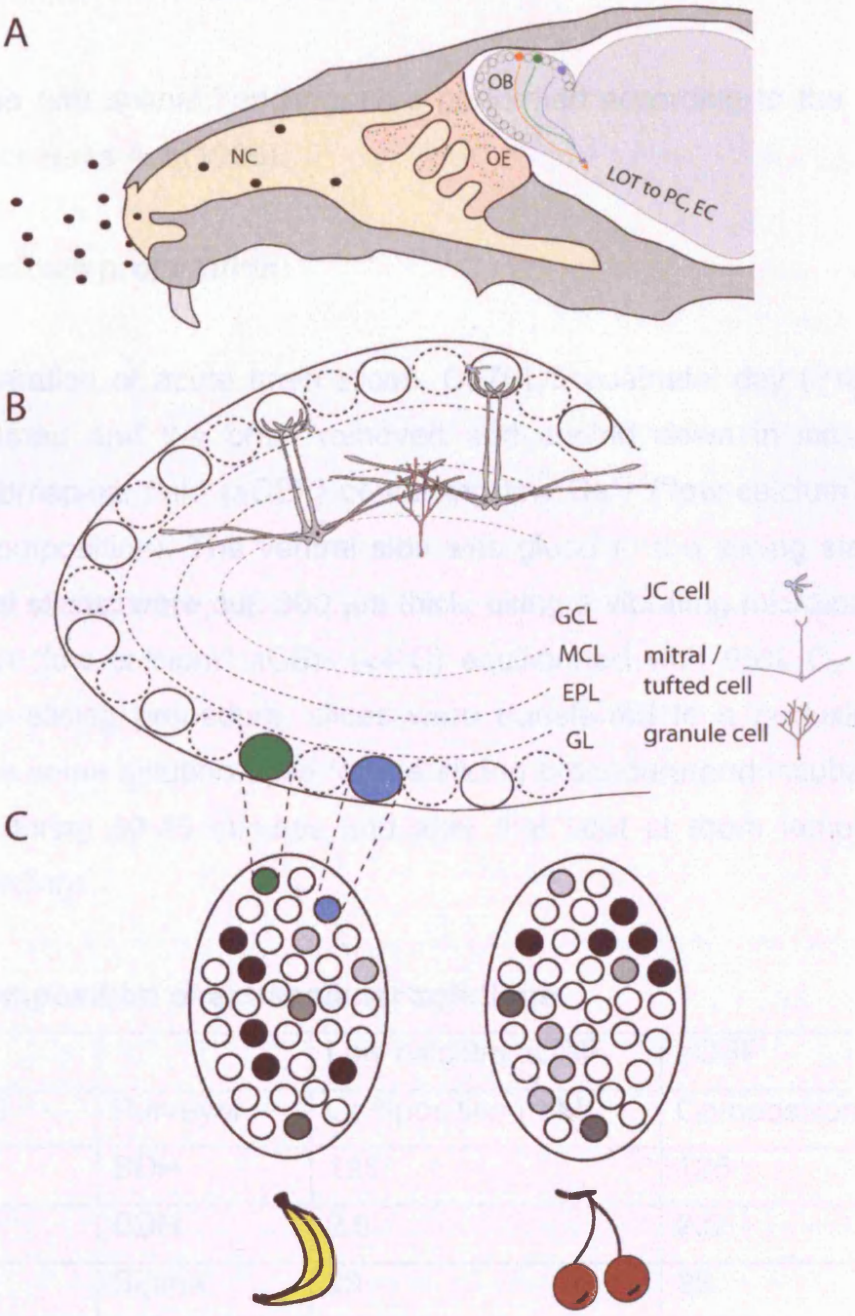

Figure 1.1 – Functional organization of the olfactory bulb
A – Airborne odor molecules inhaled into the nasal cavity (NC) during sniffing activate sensory neurons in the olfactory epithelium (OE). Functionally similar sensory neurons converge to the same glomeruli of the olfactory bulb (OB). Mitral cell axons project their axons via the lateral olfactory tract (LOT) to other regions of the brain such as the piriform cortex (PC) and the enthorinal cortex (EC).
B – Anatomical organization of the olfactory bulb. Each mitral cell commits to receiving input form a single glomerulus thus the glomerulus defines a functional module in olfactory processing. Interactions between functional modules are mediated by juxtaglomerular and granule cells (GCL- granule cell layer EPL – external plexiform layer MCL – mitral cell layer GL – glomerular layer)
C – Fuctional segregation of sensory afferents into distinct glomeruli leads to the emergence of a spatial map of glomerular activation. Different odours elicit different combinations of glomeruli

Chapter 2: Materials and methods

All procedures and animal handling were performed according to the UK Animals Scientific Procedures Act (1986).

Acute brain slices preparation:

For the preparation of acute brain slices, C57BL/6 postnatal day (P)20-P27 mice were decapitated and the brain removed and cooled down in ice cold (<4°C) artificial cerebrospinal fluid (aCSF) containing low Ca^{2+} ("low calcium" aCSF; see table 1 for composition). The ventral side was glued to the slicing stage platform and horizontal slices, were cut, 300 µm thick, using a vibrating microtome (HM 650 V, Microm) in "low calcium" aCSF (<4°C) equilibrated with 95% O_2 / 5% CO_2 . Following the slicing procedure, slices were transferred to a perfusion chamber containing the same solution used for the slicing procedure and incubated at 34°C temperature during 30-45 minutes and after that kept at room temperature until used for recordings.

Table 1 – Composition of extracellular solutions

		Low calcium aCSF	aCSF
Compound	Purveyor	Composition (mM)	Composition (mM)
NaCl	BDH	125	125
KCl	BDH	2.5	2.5
$NaHCO_3$	Sigma	26	26
NaH_2PO_4	Sigma	1.25	1.25
Glucose	BDH	25	25
$CaCl_2$	Fluka	1	2
$MgCl_2$	Fluka	2	1
pH ~ 7.4			

Whole-cell recordings:

Whole-cell recordings were performed from the somas of visually identified mitral and tufted cells using infrared differential interference contrast (IR-DIC) microscopy (Stuart *et al.*, 1993). For visualization, a Zeiss Axioscope II was used fitted with a 40x water immersion lens fitted with a Luigs and Neumann multiplier (magnifying up to 2x) coupled to a CCD camera (VX55, TILL Photonics) and a monitor (Samsung). Horizontal slices retain the layered structure of the olfactory bulb and thus cells belonging to different layers can easily be visualized and targeted for recordings in these slices. Mitral and tufted cells can be easily identified as they are the cells with the largest somas in the olfactory bulb. Mitral cells stay in a clearly demarked cell layer below the external plexiform layer (EPL) and above the densely packed granule cell layer. In several instances displaced mitral cells/tufted cells (whose somas were no more than 166 µm distant to the mitral cell layer) were also recorded in this study (7 tufted cells out of 198 total cells; no simultaneous recordings from two tufted cells are included).

For whole-cell recordings, electrodes (4-7MΩ resistance) were prepared from thick walled borosilicate glass (I.D. 0.86mm O.D. 1.5 mm; Harvard Apparatus), pulled with a 2 step puller (PC-10, Narishige) and filled with a low chloride methansulfonic acid based internal solution (see composition in table 2)

To the extracellular solution, aCSF (see composition in table1), picrotoxin was added to a final concentration of 50 µM (unless stated otherwise). The use of picrotoxin was necessary in order to isolate glutamatergic excitatory currents/potentials which could be masked by ionotropic GABA mediated signaling.

Recordings were performed at near physiological temperature ranging between 30-34 °C which was kept constant during experiments with the use of an in-flow heater (TC-324B, Warner Instruments). Drugs were diluted in the extracellular solution to

the desired concentration and flowed into the recording chamber at the same conditions as the regular aCSF (~ 4 ml/min).

Table 2 – Composition of the internal solution

Compound	Purveyor	Composition (mM)
Methanesulfonic acid	Fluka	130
KCl	BDH	7
HEPES	Sigma	10
EGTA	Sigma	0.05
Na_2ATP	Sigma	2
MgATP	Sigma	2
Na_2GTP	Sigma	0.5
Biocytin hydrochloride	Sigma	10
osmolarity	290 mOsm	
pH	7.4 adjusted with 1M KOH (Sigma)	

Pharmacology:

Ionotropic glutamate receptor blockers/antagonists were applied at the following concentrations: 3-dihydroxy-6-nitro-7-sulfamoyl-benzo (f) quinoxaline (NBQX) 10-20 µM; Naphthyl-acethyl-spermine (NAS) 20 µM. NBQX is a general AMPA receptor antagonist and was used to block AMPA receptor mediated currents/potentials. NAS is a calcium permeable AMPA receptor specific blocker and was used to dissect currents/potentials mediated by this subtype of AMPA receptors. Picrotoxin

(50 μM) was used as a general blocker $GABA_A$ mediated inhibitory currents with the intention of isolating excitatory glutamatergic transmission into mitral cells.

Data acquisition and analysis:

Whole-cell recordings were obtained using a Multiclamp 700B amplifier, filtered at 6 - 10 kHz and digitized at 10 - 20 kHz using an ITC-18 data acquisition board controlled by the Nclamp/Neuromatic package. Data was analyzed offline using Neuromatic (www.neuromatic.thinkrandom.com) software that runs within the Igor Pro (Wavemetrics) environment.

Quantification of self and lateral excitation

This work aimed to quantify the relationship between self excitation, electrical coupling and lateral excitation in pairs of mitral cells. In order to evoke self and lateral excitation single action potentials (APs) were induced by injecting current pulses of 2 ms duration and 1.6-1.8 nA in amplitude in mitral cells. Self excitation induced depolarizations are measured in the same mitral cell in which action potentials are evoked. For lateral excitation (and electrical coupling) measurements, simultaneous recordings from two mitral cells projecting to the same glomerulus are required. Action potentials were evoked in one of the cells (presynaptic) while the membrane voltage is monitored in the second cell (postsynaptic). This is considered as one connection, for each pair two presynaptic/postsynaptic arrangements (or connections) are possible. The amplitudes of self excitation and lateral excitation reported in this thesis refer to the responses evoked by a single action potential. Amplitudes were determined from an average of at least 12 events, measured at the time of the peak, which was defined as the time at which the membrane voltage reaches the maximum within a 10 ms interval beginning 0.5 ms after the end of the presynaptic current injection. In order to accomplish that, a 2 ms averaging window was centered on the time of the peak and compared with a 2 ms baseline window

that ended 1 ms before the time at which current injection (necessary for the evoking the action potential) began. The difference in membrane voltage (or current if in the voltage clamp configuration) between the two windows was quantified as being the amount of self excitation or lateral excitation. In cases where lateral excitation was smaller than 0.2 mV the peak depolarization values from at least 12 individual sweeps were statistically compared with those from the baseline window. In cases in which the membrane voltage in the second cell was not statistically different from the baseline value, lateral excitation was considered not to be detectable. To minimize contamination by voltage-dependent conductances, self-excitation was determined by digitally subtracting the action potential waveforms in the presence of AMPA receptors antagonists from the control waveforms in traces in which the baseline membrane voltage differed by less than 300 µV. Each trace was interpolated to 100 kHz and aligned to the AP peak before subtracting with an average AP obtained from the average of 4-10 single APs recorded after the perfusion of NBQX for at least 8 min (Pimentel & Margrie, 2008).

Quantification of paired pulse ratios

Paired pulse ratios of excitatory postsynaptic potentials (EPSPs) were quantified as the ratio between the amplitude of the second EPSP over the first which were evoked by two APs at various frequencies (ranging from 5-100 Hz).

Electrical coupling

For the assessment and quantification of electrical coupling between mitral cells injection of hyperpolarizing pulses (-400 to -800pA, 600ms duration) was alternated to both cells. In order to quantify electrical coupling, the ratio (coupling coefficient; CC) between the steady state amplitude of the evoked voltage deflection in the test cell to the response evoked in the pre-synaptic cell was determined. In cases where the average coupling coefficient for both directions was smaller than 0.01 the post-

synaptic hyperpolarizations were checked for statistical significance when compared to the baseline membrane potential. In all cases where the morphology of pairs could be confirmed, mitral cells projecting to the same glomerulus showed statistically significant (t-test) coupling coefficients.

Theta burst stimulation

Theta burst stimulation (TBS) consisted of 150 bursts of 5 APs at 50Hz repeated at 5Hz (750 APs, total duration 30s). The same hyperpolarizing pulses used for quantification of electrical coupling were used to monitor input resistance during the plasticity experiments. A baseline stability criterion was applied to plasticity experiments, those experiments in which the first half of the baseline was significantly different (t-test) from the second half were rejected.

Recurrent inhibition

Recurrent inhibition was evoked using a burst of 5 APs at 50 Hz and quantified by digitally subtracting the burst waveforms from those in the presence of picrotoxin (Margrie *et al.*, 2001). Similarly recurrent IPSP amplitude resulting from the burst was quantified as the difference between a 2 ms baseline window positioned before the first AP and a 2 ms detection window which was set centered on the minimum voltage of the subtracted waveform.

Analysis of anatomical parameters

Dendrotomies were carried out either indirectly (via non-specific amputation during the slicing procedure) or directly using two patch pipettes in a scissor-like motion under visual guidance. In all cases dendritic amputation was confirmed by morphological analysis. Recovery of biocytin-filled cells was carried out as described previously (Horikawa & Armstrong, 1988). Slices were fixed overnight in a

4% Paraformaldehyde (PFA) Phosphate buffered saline (PBS) solution. After washing out fixative they were permeabilized with 100% methanol at -20 °C and incubated with Vectastain ABC kit (Vector Labs) overnight at 4 °C. ABC conjugates that remained unbound were thoroughly washed with PBS which followed incubation with 3,3'-Diaminobenzidine (DAB) reagent. DAB molecules are enzimatically processed and the product upon reaction with H_2O_2 forms a dark precipitate which is visible under light microscopy. Morphologies of recovered cells were then reconstructed using the Neurolucida system (Microbrightfield Inc.) in order to allow quantification of its morphological properties.

Quantification of mitral cells morphometric properties was performed using the built-in analysis functions of Neurolucida Explorer (part of the Neurolucida System; Microbrightfield Inc.). For the quantification of mitral cell tuft morphology the soma, apical dendrite and lateral dendrites were not considered. For the quantification of lateral dendrites the soma and apical dendrite were excluded. Surface area was computed by treating dendritic segments as frusta. The three dimensional Convex Hull analysis provides an estimate of the size of the dendritic field of the tufts. It is derived from a convex polygon generated by connecting the tips of the outer dendritic processes. The surface area of the smallest convex polygon for both tufts therefore provides a measure of the space occupied by both cells within a glomerulus. For determining the predicted mean surface area for a mitral cell pair 12 single mitral cells (i.e. cells projecting to different glomeruli; non-pairs) were reconstructed and their morphologies quantified. Mean values were calculated and multiplied by a factor of two and used as the expected pair morphometry. For the dendritic proximity analysis, histograms reflecting the distance between two dendrites from different cells were obtained from Neurolucida Explorer. Histograms were generated using 0.1µm bins. The closest distance between two dendritic arbors (or dendritic proximity) was considered to be the lower limit of the first bin that contained data points for each pair of mitral cells (Pimentel & Margrie, 2008).

Chapter 3: Self excitation of olfactory bulb mitral cells

Introduction:

Conventionally in the central nervous system (CNS) chemical synaptic transmission occurs due to release of neurotransmitter from axonal boutons, activating opposing specialized regions, located in the dendrites of other neurons or target structures. However dendritic release has also been shown to occur in in the olfactory bulb and other brain regions (Margrie & Urban, 2007). Mitral cells are known to have glutamate containing synaptic vesicles located throughout the entire length of their lateral dendrites and apical dendritic tuft (Rall *et al.*, 1966; Pinching & Powell, 1971b). Upon mitral cell activation APs backpropagate into the dendrites resulting in Ca^{2+} influx (Bischofberger & Jonas, 1997; Chen *et al.*, 1997; Charpak *et al.*, 2001) which triggers the fusion of these vesicles with the dendritic membrane allowing the release of glutamate. These events have been shown to directly mediate the mechanisms of recurrent and feedforward (or lateral) inhibition in the olfactory bulb (Pinching & Powell, 1971b; Jahr & Nicoll, 1980; Aungst *et al.*, 2003; Murphy *et al.*, 2005). Glutamate release from mitral cell dendrites activates glutamate receptors expressed in the postsynaptic membranes of local interneurons which in turn leads to their activation and concomitant inhibition of the same presynaptic mitral cell and other mitral cells. Juxtaglomerular cells are responsible for mediating inhibition at the glomerular level (though they can also be driven by direct sensory input) while granule cells do so by directly releasing GABA onto mitral cells lateral dendrites (Rall *et al.*, 1966; Pinching & Powell, 1971c, b).

In addition to playing a pivotal role in shaping of the mitral cell activity via inhibitory interactions, dendritic release of glutamate has also been shown to evoke EPSP-like depolarizations into the (releasing) mitral cell's own presynaptic dendrite (Nicoll & Jahr, 1982; Isaacson, 1999; Friedman & Strowbridge, 2000; Margrie *et al.*, 2001; Salin *et al.*, 2001; Schoppa & Westbrook, 2001, 2002). This release of glutamate

activates glutamate receptors (both AMPA and NMDA) in its own membrane providing a direct form of self excitation (SE) (Nicoll & Jahr, 1982; Isaacson, 1999; Friedman & Strowbridge, 2000; Margrie *et al.*, 2001; Salin *et al.*, 2001; Schoppa & Westbrook, 2001, 2002). Previous work provides evidence that it can arise both from lateral dendrites, largely due to the activation of NMDA receptors (Isaacson, 1999; Friedman & Strowbridge, 2000; Salin *et al.*, 2001; Schoppa & Westbrook, 2002) but also from the apical tuft of mitral cells mainly mediated by AMPA receptors (Margrie *et al.*, 2001; Salin *et al.*, 2001; Schoppa & Westbrook, 2002). Clear understanding of the rules of self excitation's reliability, integration and anatomical correlates as well as its role in olfactory processing are lacking.

Results:

In acute olfactory bulb slices self excitation in mitral cells can be detected its effect quantified, using whole-cell recordings, by comparing the action potential waveform in mitral cells in the absence and presence of glutamate receptor antagonists (Figure 3.1A). The self-excitatory potential arises simultaneously with the afterhyperpolarizing potential (AHP) thus the two are overlapping which perturbs the direct inspection and quantification of self excitation. The application of glutamate receptor antagonists blocks the self-excitatory effect of glutamate and one can take advantage of this by digitally subtracting the AP waveforms recorded in the presence of the antagonist from the ones recorded in control conditions. This subtraction unmasks the self excitatory potentials and allows offline quantification. However, the action potential waveform shows a large variability in the afterhyperpolarization from trial to trial as it is visible in Figure 3.1B (n = 40). All the AP waveforms shown were recorded in control conditions (absence of NBQX) and were baselined to the resting membrane potential (V_{rest}) in order to highlight the variability of the AP waveform in the time frame that is relevant for the AHP and self excitation quantification. One explanation for such variability could be that self excitation amplitude shows a high failure rate or a high variability in amplitude

across trials while a second possibility is that this is caused by variability in the AHP directly. Given that the self excitation can only be quantified by subtraction it is crucial to understand what is the cause of such variability as subtraction of a component which is not sensitive to NBQX will lead to inaccurate quantification of self excitation.

Comparing the AHP amplitude between successive trials revealed a correlation with the resting membrane potential when the population was sampled from a broad membrane potential range (V_{rest}; Figure 3.1C1; Control -68.5 < V_{rest} < -64.6 mV; $p < 0.01$; NBQX -67/6 < V_{rest} < -66.8 mV; $p < 0.05$). The impact of V_{rest} is also evident in the traces shown in figure 3.1C2. In those traces that were recorded at similar membrane potentials the AHP waveforms are almost indistinguishable while in those that differ more substantially AHP waveforms are clearly distinct. Example traces are shown either maintaining the V_{rest} variability across traces and after being baselined to the same V_{rest} in order to highlight differences in AHP amplitude. Such impact of V_{rest} on AHP amplitude could introduce a bias in the quantification of self excitation if the subtraction is performed between traces that were recorded at different resting membrane potentials. Thus it was necessary to estimate which limiting parameters would allow accurate quantification of self excitation with minimal interference of such membrane potential induced fluctuations in the AHP amplitude. Consistent with the observation that traces that were recorded at similar V_{rest} have indistinguishable waveforms, grouping a subset of data points that differed <0.3 mV from a randomly chosen membrane potential disrupted the resting membrane potential AHP amplitude correlation (NBQX; black and dark grey dots figure 3.1C1). However when compared to the remaining more hyperpolarized (red dots) or depolarized (blue dots) they have significantly different amplitudes. Given this fact it seems that, while monitoring self excitation, it is necessary to compare only traces that were recorded at very similar V_{rest} and it seems it is possible to do so with minimal contamination if they are compared within a 0.6 mV range (± 300 μV).

An example of digital subtraction performed to quantify self excitation is provided in figure 3.2A (same cell as in figure 3.1). Subtraction of the traces recorded in the presence of the NBQX (top; red trace) from ones in control situation (top; black trace) unveils the EPSP-like waveform of self excitation (below; blue trace). As expected considering the observed sensitivity of the AHP to V_{rest} the subtracted self excitation exhibits a complementary sensitivity (figure 3.2B; $p < 0.01$). Traces that were recorded at more depolarized potentials than those used for the subtraction (i.e. those in the presence of NBQX) reveal smaller self excitation while those recorded at more hyperpolarized potentials appear to show larger self excitation. This can easily be visualized in the example traces provided (Figure 3.2C; obtained from the same traces shown in 3.1D). Traces are presented as before in order to highlight the differences in self excitation amplitude. Note that within each colour group traces shown were selected so that they differed less than 0.6 mV, confirming that within that range measurements of self excitation are reliable. In addition the correlation between self excitation amplitude and V_{rest} is no longer significant in traces recorded within a narrower range of V_{rest} (black dots Figure 3.2B; $p > 0.05$). Thus it seems that restricting self excitation quantification to traces that differ from those used for subtraction in less than ± 300 μV is an effective way of minimizing AHP induced contaminations. Using these criteria I have found that self excitation could be reliably evoked by a single action potential, had an average amplitude of 1.9 ± 0.32 mV (n=34) but showed a large variation between cells, self excitation ranging from 0.26 to 8.5 mV (figure 3.2D).

The pharmacological profile of self excitation

Recent studies have shown that both calcium permeable (CP-AMPA) and calcium impermeable (CI-AMPA) AMPA receptors coexist in the olfactory bulb (Montague & Greer, 1999; Sassoe-Pognetto & Ottersen, 2000; Sassoe-Pognetto *et al.*, 2003; Blakemore *et al.*, 2006; Ma & Lowe, 2007). They have been shown to mediate in part the olfactory nerve- mitral cell synaptic transmission and also to mediate self

excitation in external tufted cells (Blakemore *et al.*, 2006; Ma & Lowe, 2007). The use of the CP-AMPA receptor specific blocker Naphthyl-acethyl-spermine (NAS) allowed me to identify the effector receptors underlying the self-excitatory potential in mitral cells. Using the subtraction approach mentioned before I have found that NAS blocks the self-excitatory potential. The time course of this block is shown in figure 3.3A1 and average traces representative of self excitation during control conditions and bath application of NAS and NBQX are shown in figure 3.3A2. NAS bath application blocked self excitation by approximately 71% (residual was 28.6 ± 21.3% of control amplitude n=8, $p < 0.01$; Wilcoxon paired sample test; Figure 3.3B) and occluded the antagonizing effect of NBQX, thus self excitation is mediated largely by CP-AMPA receptors.

Short-term dynamics of self excitation

I next examined the short term dynamics of self excitation as this could provide clues to the integrative properties of this mode of communication in mitral cells. In order to do so I evoked pairs of APs at various frequencies and repeated these range of frequencies across various cells in order to obtain the short-term dynamics profile of self excitation. Example traces for such an experiment are shown in Figure 3.4A (left; A1-A5 corresponding to 100, 50, 20, 10 and 5Hz respectively). In order to assess the impact of the frequency of the two pulses on the amplitude of the second event, the ratio of the two, the paired pulse ratio (PPR = 2^{nd} PSP amplitude / 1^{st} PSP amplitude) was determined. The contribution of the 1^{st} PSP to the amplitude of the second due to summation was removed by subtracting the baseline. For higher frequencies self excitation showed short-term depression of its efficacy. Population PPR ratios are shown in Figure 3.4A1-5 (right; bar graphs). For 100 Hz and for 50 Hz I observed depression of self-excitation. Interestingly for frequencies of 20 and 10 Hz I observed the opposite effect with the second PSP showing facilitation instead of depression. The second event amplitude was unaffected when they occurred at a frequency of 5 Hz.

The anatomical locus of self excitation

I performed dendrotomies of mitral cell apical dendrites in order to investigate the locus and reliability of self excitation. Recovery of the morphology of mitral cells following these recordings, allowed the confirmation of the procedure. Examples of an intact (left) and a dendrotomized cell (right) are shown in Figure 3.5A. Should self excitation partially or completely arise from this locus one should find a difference in the amplitude or absence of self excitation in these cells. In fact, and consistent with previous studies in the rat (Salin *et al.*, 2001; Schoppa & Westbrook, 2001), I found that AMPA receptor mediated self-potentials are absent in mitral cells with a dendrotomized apical dendrite. Figure 3.5B (left) shows the superimposed AP waveforms of a dendrotomized mitral cell, recorded in the absence (control; back trace) and presence of NBQX (red trace). As is evident from the subtracted trace (bottom; blue trace), there is no measurable NBQX sensitive component, self excitation amplitude was on average 0.03 ± 0.04 mV (n=5, Figure 3.5B right). Furthermore and in contrast to the lack of self excitation in dendrotomized cells all morphologically identified cells with an intact dendritic arbor displayed robust self excitation, average amplitude 1.9 ± 0.32 mV (n=34, Figure 3.5B right). Figure 3.5C shows a histogram of the distribution of self excitation across the different cells, all morphologically identified intact mitral cells (black bars) displayed self excitation while dendrotomized cells, can clearly be distinguished by the lack of self excitation (white bar). This suggests that it must be the release of glutamate and activation of AMPA receptors in the apical tuft that is responsible for the observed excitatory potentials.

However one other possible explanation for the lack of self excitation could be that the amputation of the main dendritic arbor could somehow render the lateral dendrites unable to release glutamate. In this scenario the lack of self excitation

could be due to a degradation of glutamate release caused by an overall cellular unhealthiness rather than the direct amputation of its locus. In order to test for this possibility I examined whether it would be possible to evoke recurrent inhibition onto dendrotomized mitral cells. As mentioned previously release of glutamate from lateral dendrites is responsible for the activation of granule cells that in turn, by the release of GABA, evoke a recurrent inhibitory postsynaptic potential (rIPSP) in mitral cells. In order to reliably evoke rIPSPs onto mitral cells, bursts of 5 APs at 50 Hz were evoked by current injection in mitral cells. Burst waveforms recorded in the presence of picrotoxin were digitally subtracted from the control (no picrotoxin) waveforms so that the rIPSP could be revealed (Figure 3.6A; Margrie *et al.*, 2001). This approach allowed me to detect and quantify recurrent inhibition in both intact and dendrotomized cells. Figure 3.6A (left) illustrates an example of such bursts of APs recorded in an intact mitral cell, the burst recorded in control conditions (black trace), in picrotoxin (red trace) and the waveform resulting from the digital subtraction of the two, the rIPSP waveform (blue trace). In contrast to self excitation it was possible to evoke recurrent inhibition in dendrotomized cells as well as in intact cells (Figure 3.6A, right). Population data that compares the expression of self excitation and recurrent inhibition in intact and dedrotomized cells is summarized in Figure 3.6B. There was a trend (although not significant) of increased inhibition onto dendrotomized mitral cells, which could be explained by the fact that dendrotomized mitral cells had increased input resistances (Bekkers & Hausser, 2007). Nevertheless the fact that recurrent inhibition is not impaired in dendrotomized cells suggests that although glutamate release can occur from the lateral dendrites of mitral cells, no AMPA receptor mediated component can be measured at the soma. Given that fact the locus for AMPA-mediated self excitation must be in the apical tuft. Taking into consideration the fact that (intact) mitral cells always showed self excitation, one can conclude that this is an universal feature of such cells. Self excitation must contribute to olfactory processing by providing a source of depolarization at the apical tuft. In addition it also provides a source for calcium influx which could play a role in several intracellular signaling pathways.

Anatomical correlates of self excitation

Despite being ubiquitous across mitral cells the overall amplitude of the self-excitatory potential varied greatly (Figure 3.5C). Because the source of this variability is unclear I decided to investigate whether it may relate to diversity in the morphology of the mitral cells apical tufts. In order to assess whether there is an anatomical correlate for the amplitude of self excitation in mitral cells I have examined the morphology of 12 intact mitral cells and extracted information about their apical dendritic arborizations, which is summarized in figure 3.7. Figure 3.7A shows an example of one of the examined cells (c5). The apical arborization of this cell is highlighted (red circle) and its branching pattern represented in the form of a two dimensional dendrogram. This tuft had a total dendritic length of 1.39 mm (Figure 3.7B; red boxed bar c5), 46 branching nodes (Figure 3.7C; red boxed bar c5), a total surface area of 3574 μm^2 (Figure 3.7D; red boxed bar c5). I have also determined the Convex Hull polygon parameters for the tufts (surface and volume), which provides a measure of the three dimensional field occupied by the tuft. It is calculated by simulating a polygon that joins the outer perimeter of the tuft. Thus rather than quantifying the volume of the dendritic branches, it provides an idea of the space occupied by the tuft. For the example neuron (c5) these values were $16.6x10^3$ μm^2 surface area and $129.6x10^3$ μm^3 total volume. Population data for all the reconstructed Mitral cells is shown in Figures 3.7B-E with the average displayed in the darker (first) bars. On average a MC has 1.17 ± 0.14 mm length (n=12; Figure 3.7B), 38.5 ± 4.5 nodes (n=12; Figure 3.7C), 2921 ± 427 μm^2 surface area (n=12; Figure 3.7D). Convex Hull analysis was only possible for 10 of the 12 mitral cells due to a few broken dendrites that interfere with the determination of the polygon. The average convex Hull surface area was 9509 ± 1302 μm^2 (n=10; Figure 3.7E green bars) and average volume was 48 ± 11 $x10^3$ μm^3 (n=10; Figure 3.7E brown bars). I then searched for a correlation between all these parameters and the measured self excitation amplitude in the same cells. Plots for the relationship

between each morphometric parameter and self excitation amplitude are displayed in figures 3.8A-E. I found no correlation between any of these morphometric properties of mitral cell tufts and self excitation amplitude (figures 3.8A-E) suggesting that more subtle features of mitral cell tuft determine self excitation strength.

Discussion:

All together these results show that self-excitation is a ubiquitous trait of olfactory bulb mitral cells, though variable in strength, it likely contributes substantially to the depolarization of the apical tuft during mitral cell activation. Previous studies had elaborated on two different sources for these depolarizations, an NMDA receptor mediated component which arises exclusively from the lateral dendrites; and a somewhat weaker component mediated by AMPA receptors arising mainly from the apical tuft (Isaacson, 1999; Friedman & Strowbridge, 2000; Margrie *et al.*, 2001; Salin *et al.*, 2001; Schoppa & Westbrook, 2001, 2002). These studies proposed various roles for the relevance of such an event in the patterning of mitral cell activity. Among these are facilitation of rebound spikes in the absence of inhibition, overall increase in AP discharge frequency within bursts and synchronization of supra and subthreshold activity between mitral cells (Isaacson, 1999; Friedman & Strowbridge, 2000; Margrie *et al.*, 2001; Salin *et al.*, 2001; Schoppa & Westbrook, 2001, 2002). My data shows that the AMPA receptor component of this form of communication is reliable and capable of providing substantial depolarization onto mitral cells after a single AP. Its mediation by CP-AMPA receptors in addition to a depolarizing action self excitation also provides the tuft with a source of calcium influx, though the functional implications of such calcium influx remain unexplored. CP-AMPA receptors have been shown to be crucial in some forms of synaptic plasticity (Mahanty & Sah, 1998) and in the retina, where expressed pre-synaptically, they can directly mediate transmitter release (Chavez *et al.*, 2006; Cull-Candy *et al.*, 2006). Also it has been proposed that calcium influx in mitral cells

regulates the activation of SK channels which in turn have been implicated in modulating mitral cells excitability (Maher & Westbrook, 2005). It is unclear at this stage what role such calcium influx (via CP-AMPA receptors) might play in olfactory processing, but given the fact that mitral cell dendrites act both as transmitters and receptors of synaptic transmission it would be exciting to investigate whether self excitation plays a regulatory role in dendritic release and/or plasticity.

I have observed that self excitation is reliably evoked by a single action potential, in these experimental conditions it is the backpropagating action potential that provides the source of depolarization and calcium required for release to occur. One possibility is that incoming sensory input could provide with depolarization enough to locally activate voltage sensitive calcium channels and consequently induce release at the tuft in the absence of a backpropagating action potential. In addition olfactory nerve-mitral cell synapses are also know to express CP-AMPA and NMDA receptors which could further contribute with a calcium source. This could dissociate processing occurring at the glomerular level from somatic output and could impact substantially on lateral inhibition mediated via juxtaglomerular cells as well as on the timing of mitral cell discharge.

Mitral cell activity is tightly linked to the odor sampling rhythm (sniffing cycle) by the means of subthreshold oscillations which are driven by the respiration cycle. This subthreshold activity has been proposed to play roles in mitral cell synchrony, spike precision and synaptic integration and a single sniff is thought to play a "quantal" role in olfactory processing. *In vivo,* upon odour presentation mitral cells tend to fire bursts of a few APs at a preferred frequency of about 50-60 Hz occurring around the peak of the respiration-locked oscillation (Margrie & Schaefer, 2003). I have shown that self excitation in mitral cells shows a dynamic sensitivity to frequency. This form of communication expresses short term dynamics of its efficacy solely when events occur within time intervals shorter than 200 ms (approximate duration of the sniff cycle), thus for sensory input that presumably would be integrated within

the same sniff cycle. Paired pulse experiments revealed that at higher frequencies than that of the respiratory rhythm, self excitation amplitude can be facilitated or depressed in a frequency dependent manner. For frequencies between 50 and 100 Hz self excitation expressed short term depression. In contrast, for lower frequencies (10-20 Hz) the opposite was observed, the second PSP became facilitated and therefore its potential contribution for additional AP discharge increased. The observed switch from depression to facilitation could reflect post-synaptic properties of self-excitation. At higher frequencies receptor desensitization/occupation could be the major source of depression in the response and thus masking a possible increase in glutamate, while at slower frequencies the two events would be separated enough to enable the receptors to exhibit a facilitated response. Facilitation could be due to presynaptic increase in release probability or a use-dependent relief of polyamine block. The potency of self excitation is diminished while mitral cells discharge APs at higher frequencies and enhanced at lower frequencies suggesting it could facilitate a normalization of the overall rate of AP firing assisting the enforcement of a fixed rate of spiking in mitral cells. If sensory drive is sufficient to evoke higher frequency spiking, self excitation potency reduces its contribution for mitral cell depolarization. Should sensory drive be insufficient to evoke robust mitral cell firing then self excitation could provide a compensatory mechanism by having an enhanced contribution to mitral cell depolarization. This contrasts with the short term dynamics of NMDA mediated self excitation observed in rat acute olfactory bulb slices, in which a frequency dependent facilitation was observed (Salin *et al.*, 2001). This suggests that these forms of self-excitation might serve different roles in olfactory processing as they express different integrative dynamics. In acute slices the link to the sniff cycle is obviously lost but strong excitatory spontaneous input is present into mitral cells which can cause several millivolt fluctuations in the membrane resting potential (which are enhanced in the presence of picrotoxin). *In vivo,* due to subthreshold oscillations it is likely that, within a sniff cycle, self excitation occurs at different

membrane potentials. Thus in these conditions the AHP is likely to be modulated which might synergize or counteract self excitation.

Previous works had favored the use of the voltage clamp configuration in order to investigate self- excitatory currents in mitral and tufted cells (Aroniadou-Anderjaska *et al.*, 1999; Isaacson, 1999; Schoppa & Westbrook, 2001; Ma & Lowe, 2007) although in some instances current clamp configuration was also used (Margrie *et al.*, 2001; Schoppa & Westbrook, 2002; Christie & Westbrook, 2006). The experiments described here were performed in current clamp and with the use of a single action potential. I have found that in these conditions membrane potential jitter impacts on the quantification of self excitation. Given this fact the voltage clamp configuration offers the advantage of eliminating such jitter. The use of current clamp however allows a more physiological experimental setup enabling the simultaneous assessment of electrical coupling. In this configuration an AP is elicited which results in calcium influx with a particular dynamics allowing for the release of neurotransmitter. Altering the temporal properties of the calcium influx by abolishing or interfering with the AP waveform (which would occur in voltage clamp) could alter the release properties of mitral cells and thus likely affect self-excitation properties (Wheeler *et al.*, 1996). This was particularly important given that I was interested in evaluating the reliability of self excitation. Another limitation of the subtraction procedure in general is that any jitter occurring in the action potential will affect the quality of the subtraction. In order to avoid action potential jitter induced errors, action potentials were carefully aligned to the peak prior to the subtraction procedure (see methods for details).

It is important to note that changes in input resistance due to the addition of glutamate antagonists could also interfere with the quantification of self excitation. The conductances giving rise to the self-excitatory potential occur simultaneously with those giving rise to the AHP (hence the need of a subtraction approach). Due to the abolishment of any AMPA receptor induced conductance it is possible that

the input resistance increases in the presence of antagonists. This would cause an increase of the impact of currents in general on the membrane potential. For instance an apparent increase in the AHP due to increased input resistance would ultimately lead to an over-estimation of self-excitation amplitude.

Previous studies in the rat had shown that the AMPA receptor component of self excitation occurs mainly at the apical tuft but also from the lateral dendrites, though with much less potency than NMDA dependent self excitation (Salin *et al.*, 2001). My results suggest that rather self excitation is exclusively confined to the tuft and that dendrotomized cells although devoid of self excitation are still capable of evoking recurrent inhibition and therefore capable of releasing glutamate and of integrating synaptic input. This supports the idea that the AMPA mediated component is exclusive to the apical tuft.

I have also found that self excitation is a reliable hallmark of mitral cells occurring in all cells with an intact apical arborization. Still despite being a ubiquitous feature of mitral cells, self excitation varied greatly in potency across cells. The origin of this variability remains unknown. I have compared several gross morphometric parameters of the mitral cells apical tufts in search for the determinant of self excitation potency. Mitral cell tufts showed diversity in their morphologies but despite this I have found no evidence that the quantified anatomical features determine self excitation potency. This suggests that more fine approaches of mitral cell morphometry analysis, such as electron microscopy (Denk & Horstmann, 2004), are necessary in order to find the anatomical substrate of self excitation. Could it simply arise from spill over from synapses formed with other cells or rather that a more specific structure is involved? Mitral cell tuft processes from the same cell very closely appose each other which could easily provide the opportunity for the formation of a "self-synapse" or simply for spill over to occur from the abundant glutamate containing vesicles. Self excitation is in general quite potent and fast suggesting it will likely contribute to olfactory processing.

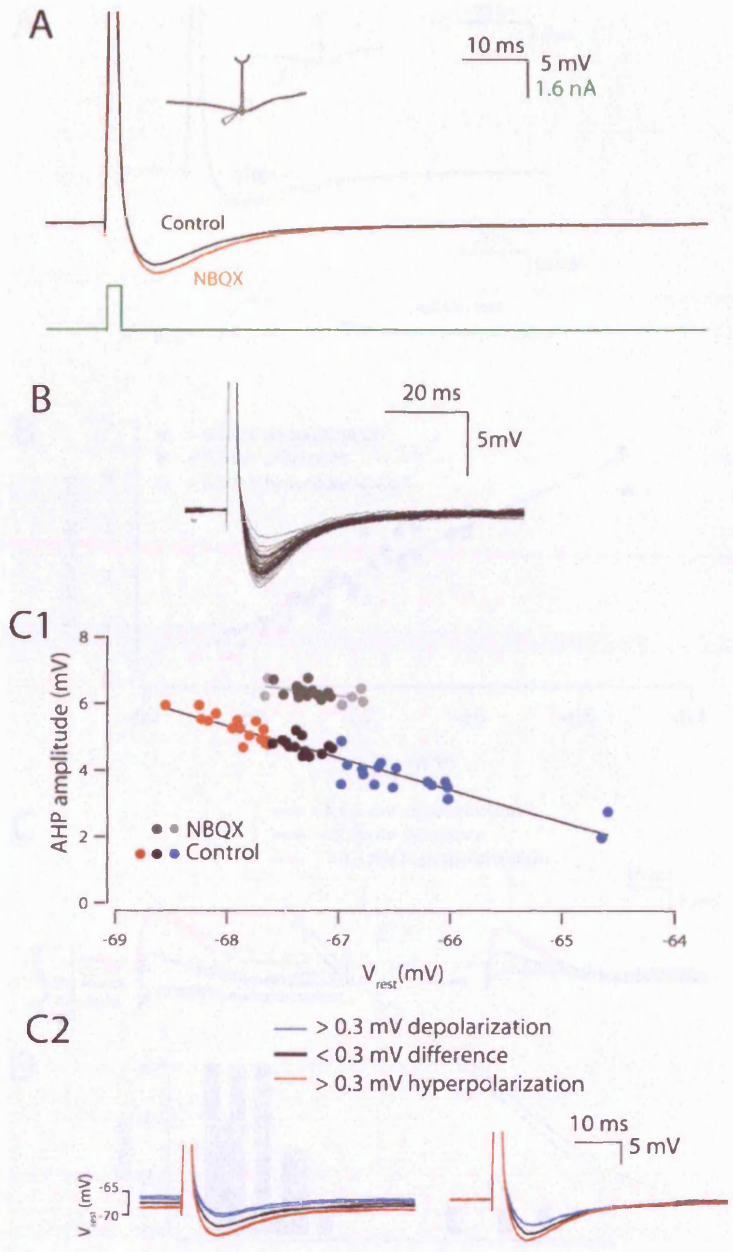

Figure 3.1- Quantifying self excitation in olfactory bulb mitral cells
A - Comparing APs recorded in the absence (control) and presence of NBQX uncovers self excitation in mitral cells. The green trace represents the brief current injection pulse (2 ms) used to evoke a single action potential in mitral cells.
B - Variability of the AP waveform for the same mitral cell (n=40 APs).
C1 - The afterhyperpolarizing potential (AHP) amplitude correlates with the resting membrane potential.
C2 - Superimposed waveforms of APs recorded at various resting membrane potentials (Vrest). APs in which the membrane potential differed by less than 0.3 mV have very similar AP waveforms (same colors) while those APs in which Vrest differed more than 0.3 mV can be clearly distinguished (blue and red traces when compared to the black traces). AP color refers to the (right) same APs have been baselined to the same value to highlight differences in AHP amplitude.

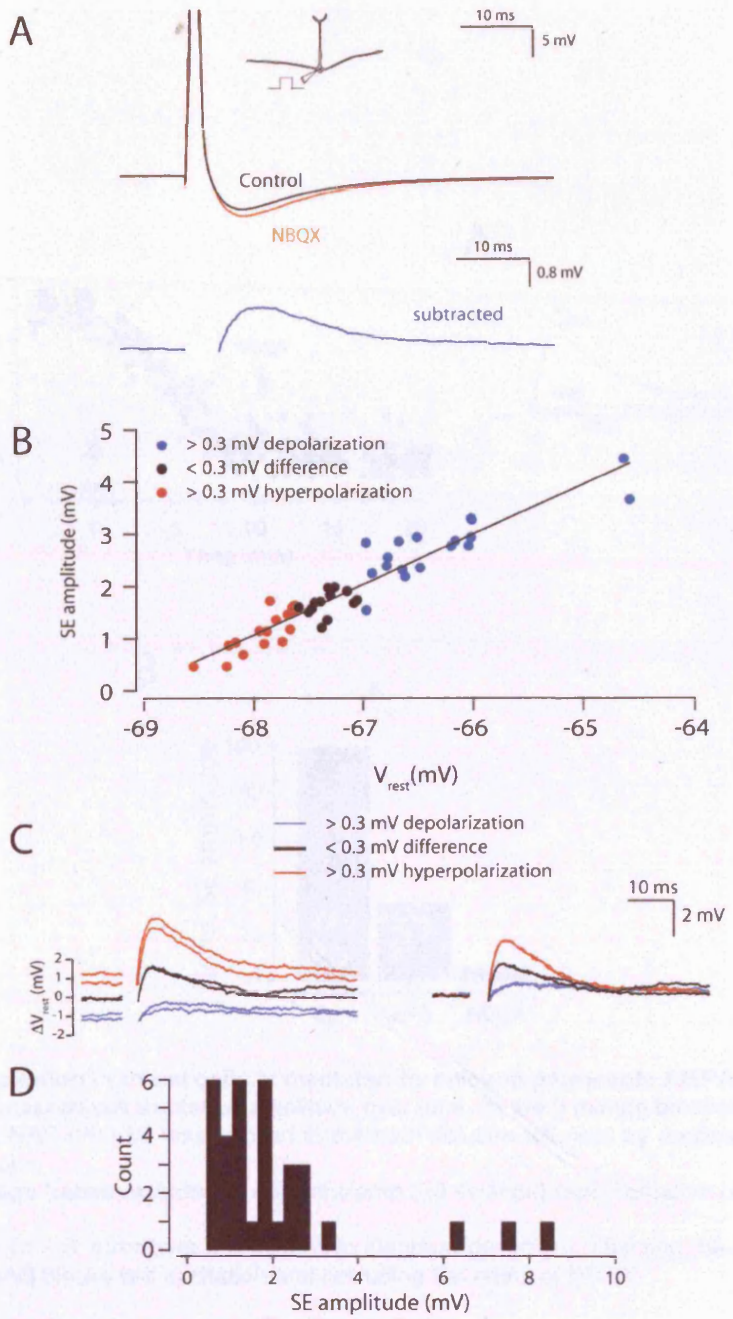

Figure 3.2- Self excitation of olfactory bulb mitral cells

A - Quantification of self excitation by digital subtraction of AP waveforms. (Top) Superimposed AP waveforms (average of 30 traces) recorded in current clamp configuration in the presence of glutamate antagonists (NBQX; red trace) and in control conditions (black trace). Digital subtraction of the two unveils the EPSP-like waveform of the self excitatory potential (below; blue trace)

B - Self excitation amplitude correlates with the resting membrane potential (Vrest; $p < 0.01$) for the whole range potentials (all data points) but not for data points that differ less than 300 µV (black dots; $p > 0.05$)

C - Superimposed waveforms resulting from subtracted traces recorded at various resting membrane potentials (Vrest). Traces in which the membrane potential differed by less than 0.3 mV have very similar amplitudes (same colors) while those in which Vrest differed by a greater amount can be clearly distinguished (blue and red traces when compared to the black traces). (Right) Color labeling refers to the same traces as in left which have been baselined to Vrest in order to highlight differences in self excitation amplitude.

D - Histogram representing the variability of self excitation potency in olfactory bulb mitral cells

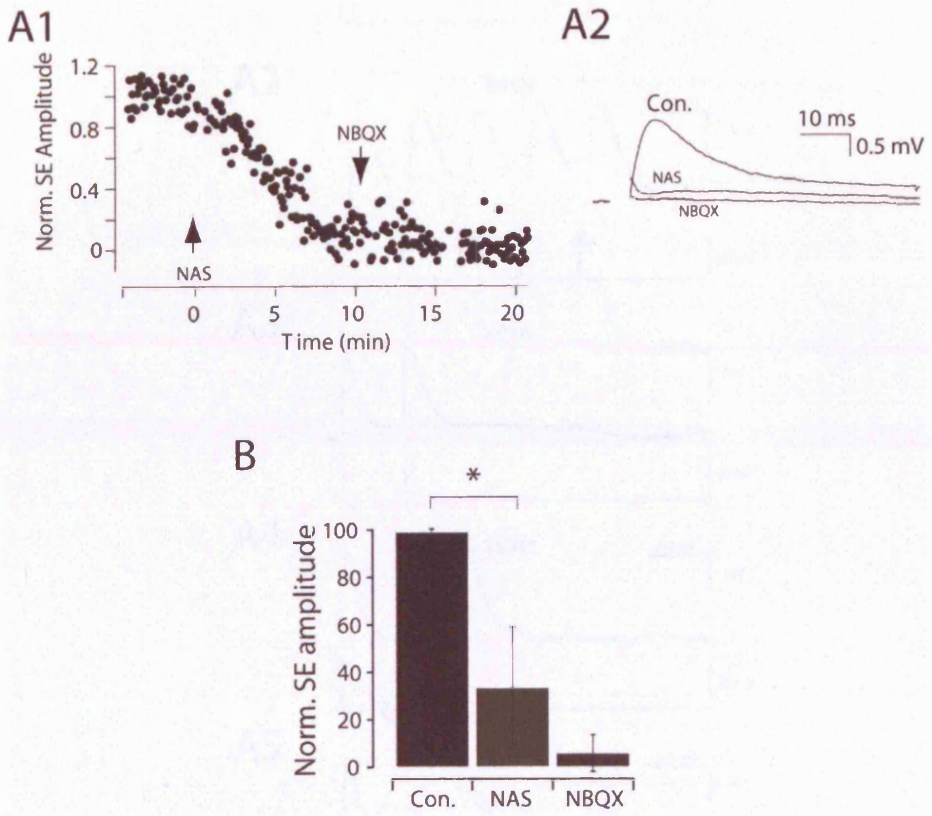

Figure 3.3- Self excitation in mitral cells is mediated by calcium permeable AMPA receptors
A1 - Plot of the normalized self excitation amplitude over time. At the 0 minute timepoint the specific CP-AMPA receptor antagonist NAS (20 μM) was applied to the bath solution followed by application of NBQX (20 μM) at the10 minute timepoint.
A2 - Example average traces recorded in current clamp (30 sweeps) representative of the control period, NAS and NBQX.
B - Population data (n = 8; error bars correspond to standard deviation) reflecting the effect of NAS and NBQX on self excitation. NAS blocks self excitation and occluding the effect of NBQX.

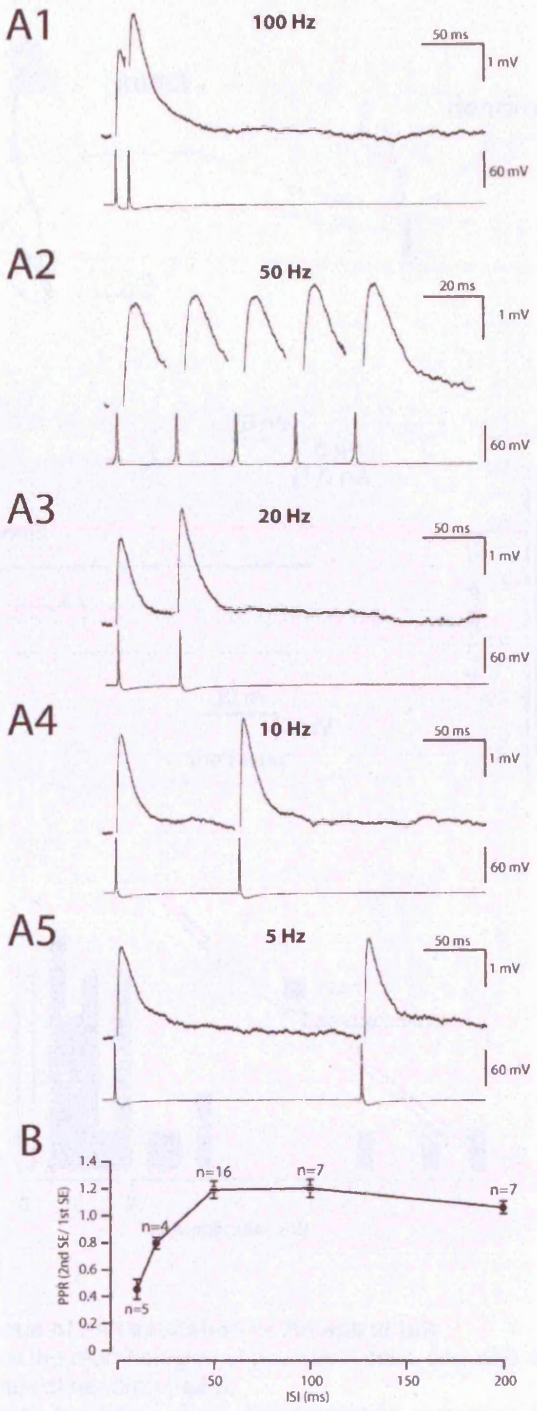

Figure 3.4- Short-term dynamics of self excitation
A1-5 - Dependence of the inter-stimulus frequency on the short-term dynamics of SE. Two APs were evoked at varying frequencies (A1-5: 100, 50, 20, 10 and 5Hz respectively). Averaged traces (left; ~20 traces) are shown to illustrate the differential integration of self excitation as a consequence of stimulus frequency (all examples except the 50 Hz trace are from the same cell).
B - Population data summarizing the short-term dynamics profile of self excitation

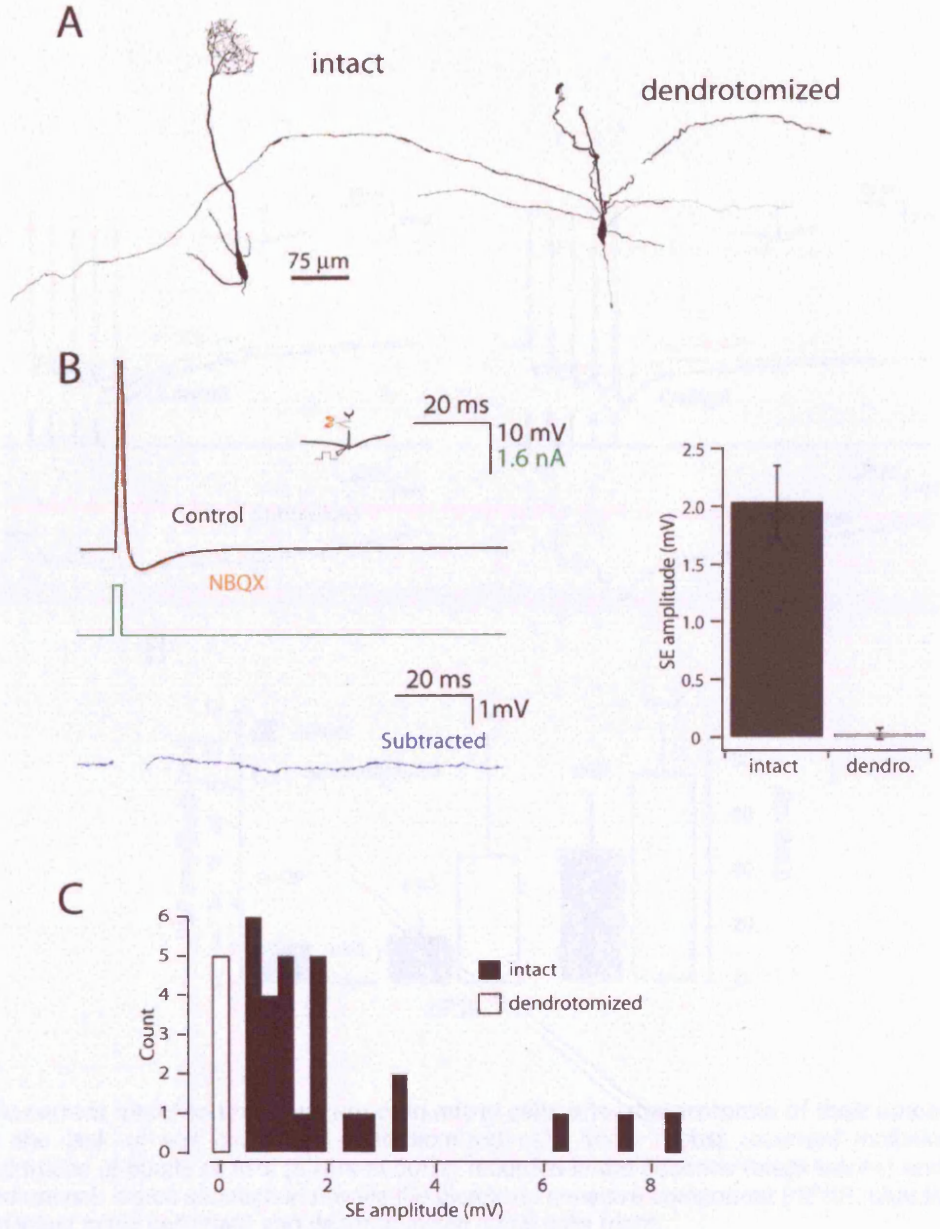

Figure 3.5- The anatomical locus of self excitation is the apical tuft
A - Neurolucida reconstrutions of the morphologies of two mitral cells, one with an intact apical arborization (left) and one with a dendrotomized apical dendrite (right).
B - Dendrotomized cells lack self excitation. (Left) Superimposed averaged AP waveforms recorded on the absence (control; black) and presence of NBQX (red). Digital subtraction of the two is shown below. (Right) Population data of the average self excitation amplitude observed in morphologically identified intact mitral cells in comparison to dendrotomized cells (n=29; error bars represent SEM).
C - Histogram plot showing the distribution of self excitation in morphologically confirmed mitral cells. All intact mitral cells (black bars; n = 29/29) displayed measurable self excitation while all dendrotomized mitral cells lacked a measurable self excitatory potential (white bar; n = 5/5).

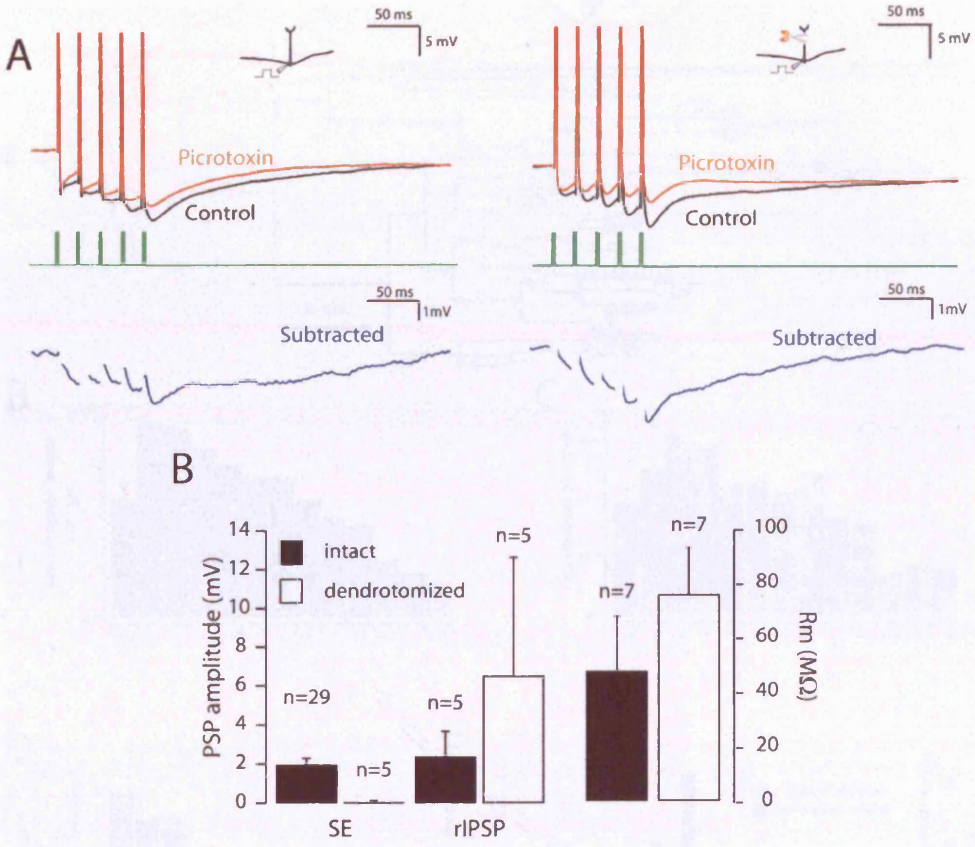

Figure 3.6- Recurrent inhibition remains intact in mitral cells after dendrotomie of their apical tufts
A - Despite the lack of self excitation, dendrotomized cells show robust recurrent inhibition. Averaged superimposed traces of bursts of APs (5 APs at 50Hz) recorded in the absence (black traces) and presence of picrotoxin (red traces), digital subtraction unveils the picrotoxin sensitive component (rIPSP; blue trace) which is robust in both intact mitral cells (left) and dendrotomized mitral cells (right).
B - (left) Plot comparing the amplitude of self-excitation and recurrent inhibition (rIPSP) and the input resistance for intact cells and dendrotomized cells. Resting membrane potential was -55.0 ± 4.9 mV (control, n = 5) and -51.4 ± 3.0 mV (dendrotomized, n = 5; $p > 0.05$ Mann-Whitney test; bars correspond to standard deviation).

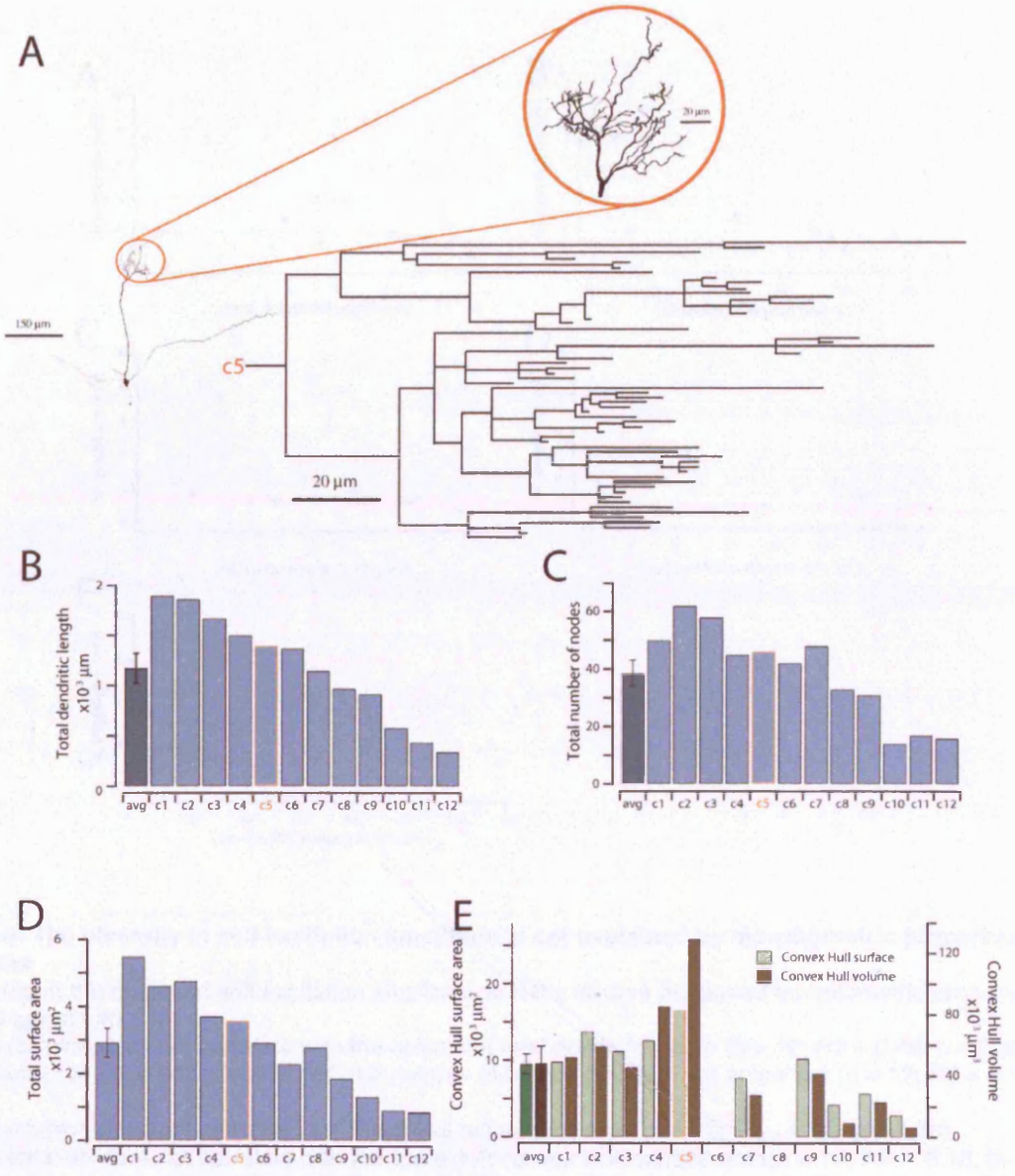

Figure 3.7- Morphometric parameters of olfactory bulb mitral cells apical tufts

A - Morphometric characteristics of mitral cells are quantified. Neurolucida reconstruction of one mitral cell of which the apical tuft is highlighted (top; inside the red circle) and the dendrogram of the same tuft is provided (below).

(B-E) Bar graphs illustrating the variability of morphometric parameters across mitral cells.

B - Total dendritic length of the apical tufts of mitral cells (n = 12)

C - Total number of nodes on the apical tufts of mitral cells (n = 12)

D - Total surface area of the apical tuft of mitral cells (n = 12)

E - Convex Hull surface area (green bars) and volume (brown bars) of mitral cell apical tufts (n = 10). For all graphs average is shown in the darkest bars and error bars correspond to SEM; example tuft (c5) shown in 3.7A is highlighted with red frames.

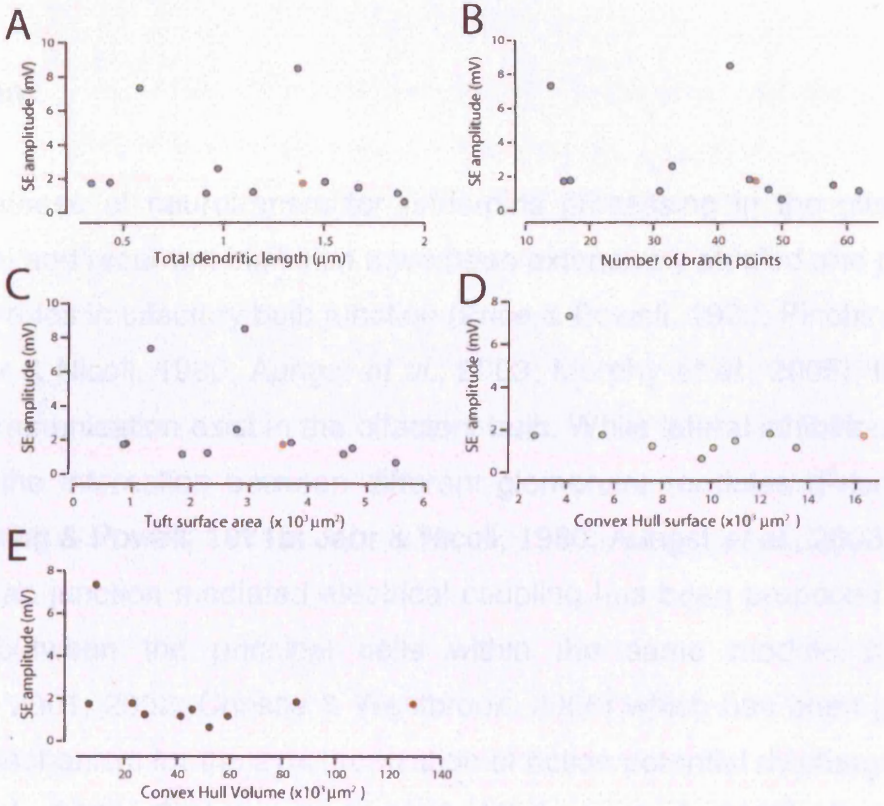

Figure 3.8- The diversity in self excitation amplitude is not explained by morphometric properties of the apical tufts

Relationship of the observed self excitation amplitude with the diverse measured morphometric properties of the mitral cell apical tufts

A - Self excitation does not correlate with the apical tuft total dendritic length (n = 12; R2 = 0.49, p = 0.48)

B - Self excitation does not correlate with the number of branch points of the apical tuft (n = 12; R2 = 0.10, p = 0.29)

C - Self excitation does not correlate with the apical tuft surface area (n = 12; R2 = 0.08, p = 0.36)

D - Self excitation does not correlate with the apical tuft convex Hull surface area(n = 10; R2 = 0.18, p=0.21)

E - Self excitation does not correlate with the apical tuft convex Hull volume (n = 10; R2 = 0.21, p = 0.13

Chapter 4: Lateral communication between intraglomerular pairs of mitral cells

Introduction:

Dendritic release of neurotransmitter underpins processing in the olfactory bulb. While lateral and recurrent inhibition have been extensively studied and proposed to play pivotal roles in olfactory bulb function (Price & Powell, 1970; Pinching & Powell, 1971b; Jahr & Nicoll, 1980; Aungst *et al.*, 2003; Murphy *et al.*, 2005), less studied forms of communication exist in the olfactory bulb. While lateral inhibition provides a means for the interaction between different glomerular modules (Price & Powell, 1970; Pinching & Powell, 1971b; Jahr & Nicoll, 1980; Aungst *et al.*, 2003; Murphy *et al.*, 2005) gap junction mediated electrical coupling has been proposed to mediate excitation between the principal cells within the same module (Schoppa & Westbrook, 2001, 2002; Christie & Westbrook, 2006) which has been proposed to provide a mechanism for the synchronization of action potential discharge (Schoppa & Westbrook, 2002; Christie *et al.*, 2005; Migliore *et al.*, 2005). Such electrical coupling has been shown to be mediated by Cx36 containing gap junctions which is the most abundant connexin in the olfactory bulb (Christie *et al.*, 2005).

Electrical transmission contrasts with chemical transmission in that it does not rely on a given neurotransmitter molecule and postsynaptic reception machinery but rather on the direct "electrical continuity" of the coupled cells provided by gap junction transmembrane channels (Hormuzdi *et al.*, 2004). Therefore flow can occur in both directions (unless in very extreme cases in which very large differences exist in the input resistances of the coupled cells). Furthermore in the case of electrical synapses there is no ion selectivity, the same synapse can be both excitatory and inhibitory depending on the excitatory/inhibitory drive into each cell (Bennett & Zukin, 2004; Hormuzdi *et al.*, 2004; Sohl *et al.*, 2005). By means of rapidly transferring subthreshold activity between cells, gap junctions act as normalizing

agents balancing excitation and inhibition of interconnected ensembles (Bennett & Zukin, 2004; Hormuzdi *et al.*, 2004; Sohl *et al.*, 2005).

Glutamate release from mitral cell dendrites, as discussed in the previous chapter, provides mitral cells with self-excitation in the form of an EPSP-like depolarization (Margrie *et al.*, 2001; Salin *et al.*, 2001; Schoppa & Westbrook, 2002). Simultaneous recordings from intraglomerular pairs of mitral cells have also revealed that similar EPSP-like depolarizations can be measured in the intraglomerular partners (Schoppa & Westbrook, 2002; Urban, 2002). Due to a lack of electron micrographic evidence in support of mitral cell - mitral cell synapses (Price & Powell, 1970; Pinching & Powell, 1971b; Kosaka & Kosaka, 2005b) lateral transmission is thought to be solely due to gap junction –mediated electrical transfer of SE. In such a scenario, gap junctions located in the tuft, proximal to the source of self excitation (Salin *et al.*, 2001) are thought to electrically transmit self excitation throughout the mitral cell network. While this form of electrically-mediated lateral transmission has been shown to facilitate spike synchronization across the glomerular network (Schoppa & Westbrook, 2001, 2002; Christie *et al.*, 2005; Christie & Westbrook, 2006) the precise relationship between SE, electrical coupling and lateral communication between mitral cells is not yet understood.

Results:

Distribution of electrical coupling between mitral cells

Several lines of research have brought to light the existence of gap junctions in the olfactory bulb and in particular that these mediate electrical signaling between mitral cells (Schoppa & Westbrook, 2001, 2002; Kosaka & Kosaka, 2003; Hormuzdi *et al.*, 2004; Kosaka & Kosaka, 2004). In order to investigate the rules and connectivity of this form of communication, I performed simultaneous whole-cell recordings from pairs of mitral cells belonging to the same glomerulus. Electrical coupling can be

assessed by injecting a large hyperpolarizing pulse in one of the cells while recording the membrane voltage in the second cell (cell A and cell B respectively Figure 4.1A bottom left). In cases in which the two cells were coupled a much smaller hyperpolarization could be measured in the second cell (cell B Figure 4.1A bottom left). The onset and offset of the transmitted hyperpolarization followed those of the injected pulse (see dashed lines, Figure 4.1A bottom) although its amplitude was markedly reduced. The ratio between the two amplitudes, coupling coefficient (CC) can be used as a measure of the strength of the connection. If coupling was present in one connection exchanging the cell to which the pulse was injected and the coupled voltage deflection recorded from always resulted in a measurable transmitted hyperpolarization (injecting to cell B and recording cell A Figure 4.1A bottom right). Thus electrical coupling between mitral cells was always bi-directional although often the coupling efficiency was different for the two directions (CC ranging from 0.003 to 0.23, mean 0.058 ± 0.048 n = 133; Figure 4.1A). As previously described gap junctions act as low pass filters and therefore there was virtually no coupling of the fast rising phase of the action potentials. Nevertheless in some situations (10/67 pairs) and, usually while in conditions that block most excitatory and inhibitory (ionotropic) inputs, it was possible to observe the transfer by electrical coupling of the afterhyperpolarization potential that follows action potentials (Figure 4.1B).

Previous studies in the rat had suggested that this form of communication occurs exclusively among those mitral cells that project to the same glomerulus (i.e. intraglomerular; Schoppa & Westbrook, 2001). Similarly in all cases where gap junction coupling was observed and histological analysis carried out, I observed a perfect correspondence between coupling and the glomerular location of the dendritic tufts (Figure 4.2A top left). An example reconstruction of such a pair and the pulses used to assess and quantify electrical coupling are shown in figure 4.2A (left). In contrast in no case did I observe electrical coupling between simultaneously recorded mitral cells whose apical dendritic tufts belonged to

different glomeruli (i.e. extraglomerular pairs; n = 14/14, Figure 4.2A right and Figure 4.2B white bars n = 5/5). Thus in addition to being always bi-directional, gap junction mediated coupling is exclusive and ubiquitous to intraglomerular mitral cells. Despite this fact the efficiency of these connections was highly variable across different pairs of mitral cells as is evident in Figure 4.2B (black bars).

While in the presence of Picrotoxin (or other $GABA_A$ blockers) rat mitral cells have been shown to receive spontaneous long lasting depolarizations (LLD's) which originate within the tuft (Carlson *et al.*, 2000). It was found that cells sharing the same glomerulus displayed highly synchronous LLD's. I found similar spontaneous events in recordings from mitral cells. Example traces for LLD's are shown in Figure 4.2C (top). As in the rat (Carlson *et al.*, 2000), LLD's in mice mitral cells were highly synchronous, as depicted in the example traces (Figure 4.2C, top left) and confirmed by the prominent peak at $\Delta t=0$ of the cross-correlation between two traces (n = 4/4; Figure 4.2C, bottom)

Distribution of lateral transmission between mitral cells

Previous studies have reported that the occurrence of an action potential in a given mitral cell would result in depolarizations that resemble EPSPs (figure 4.3A top) in their intraglomerular partners (Schoppa & Westbrook, 2002; Urban & Sakmann, 2002). Although such depolarizations have been shown to be sensitive to blockers of the AMPA and NMDA subtypes of ionotropic glutamate receptors, they have been interpreted as being due to lateral transmission of self excitation via gap junctions rather than direct synaptic transmission. Using simultaneous whole-cell recordings from pairs of mitral cells (n = 99) I found that while evoking an action potential in one of the mitral cells and recording membrane voltage in the other cell occasionally I would observe lateral transmission. Although lateral transmission was observed in the majority of cases (123/198 connections; Figure 4.3A top and 4.3B; average amplitude 0.53 ± 0.05 mV n = 123 connections) it was not measurable in a

substantial number of connections (absent in 75/198 potential connections, Figure 4.3A bottom). Therefore, unlike electrical coupling, lateral transmission is not a universal feature of intra-glomerular mitral - mitral cell communication. Given that lateral transmission appeared to be less reliable than electrical coupling I sorted pairs into three possible categories: those that expressed lateral transmission in both connections (bi-directional 39/99 pairs; Figure 4.3B right, green), in a single connection (uni-directional 45/99 pairs; Figure 4.3B right, blue) or in neither of the two possible directions (neither direction 15/99 pairs; Figure 1B right, red). In all the cases where lateral transmission was absent, the co-localization of mitral cell tufts to the same glomerulus was confirmed either anatomically (n = 6) or by the existence of electrical coupling (n = 9) since this, as shown previously, is a reliable hallmark of intraglomerular mitral cells (figure 4.2B). The strength of this form of lateral transmission was also highly variable across different pairs as shown in figure 4.3C.

The relationship between self excitation, electrical coupling and lateral transmission

Since lateral transmission between mitral cells has been proposed to be due to the propagation of self excitation via gap junction-mediated electrical coupling I next investigated directly the relationship between the degree of electrical coupling and the strength of lateral transmission. As shown in the previous chapter self-excitation is ubiquitous in mitral cells and electrical coupling provided bi-directional connections between all mitral cells that belong to the same glomerulus, whereas strikingly lateral excitation does not follow such reliability (Figure 3.4A). Using a single action potential I found that the degree of gap junction coupling between pairs of mitral cells projecting to the same glomerulus did not predict the strength nor the likelihood of expression of lateral transmission ($R^2 = 0.002$ $p > 0.5$, $n = 128$; Figure 4.4A).

One obvious potential explanation for the lack of correlation between electrical coupling and lateral transmission is that amplitude of self excitation determines the extent of lateral transmission. If self excitation is the primary source of lateral transmission variability then it together with the coupling coefficient of a given pair should predict the amplitude of lateral transmission between mitral cells. Where possible I therefore quantified self excitation, the coupling coefficients and lateral transmission for the same mitral - mitral cell connections (n = 10; Figure 4.4B). While some connections showed a modest amount of self excitation and coupling with robust lateral transmission, others showed small or no detectable lateral transmission despite similar degrees of coupling and moderate self excitation. Overall I found no correlation between self excitation, the degree of coupling and lateral transmission (Figure 4.5, $R^2 = 0.11$ $p > 0.3$, n = 10). This lack of correlation hints at the existence of an alternative form of transmission between mitral cells.

Tuft morphometry and lateral transmission

I then set out to investigate the possible locus and source of the variability in the amplitude of lateral transmission across different connections. The fact that lateral transmission occurs solely in intraglomerular pairs hints that it might occur within the glomerulus. Alternatively, despite being exclusive to intra-glomerular partners, it could occur via the lateral dendrites. To explore these possibilities, I reconstructed the tufts and lateral dendrites of a subset of the intraglomerular pairs of mitral cells (n = 5). This subgroup of mitral cells showed lateral transmission in at least one direction and a mean amplitude representative for the recorded population (reconstructed laterals mean 0.32 ± 0.14 mV n = 10 connections *vs.* recorded population mean 0.33 ± 0.04 mV n = 195, $p > 0.9$; Figure 4.5A top left). Unsurprisingly I found that in the tuft dendritic processes are often in very close opposition (mean minimum distance 0.32 ± 0.07 µm n = 5; filled dots Figure 4.5 top right) while in contrast lateral dendrites were typically far more distant (closest distance ranging from 1 - 6.5 µm; mean minimum distance 2.98 ± 1.99 µm n = 5;

empty circles Figure 4.5 top right). This finding suggests that lateral transmission, as expected is more likely to occur between apical tufts rather than between the lateral dendrites of intraglomerular mitral cells (Pimentel & Margrie, 2008).

Given that the presence and amplitude of lateral transmission was considerably variable across mitral cell pairs, one further explanation might be that its extent and efficacy is correlated with the intactness of the apical tuft/s which can easily be compromised by the slicing procedure. I therefore compared the amplitude of lateral transmission within a random selection of pairs (n = 18) with several of their morphometric parameters, which included the number of branch points, total membrane surface area and the convex Hull surface. These morphological data sets provide a measure of the space occupied by the combination of the two dendritic tufts (see chapter 3). They were then sorted into the previously mentioned categories -bi-directional, uni-directional or neither - defined by the existence and directionality of lateral transmission (Figure 4.6A, B; Pimentel & Margrie, 2008).

Analysis of variance failed to reveal a relationship between the incidence of lateral transmission and the macroscopic features of the mitral cell pairs tufts (branch points $F_{(2,15)} = 0.97$, $p > 0.4$; tuft surface area $F_{(2,15)} = 0.99$, $p > 0.3$; convex Hull surface $F_{(2,15)} = 0.27$, $p > 0.7$). There was a lack of correlation between lateral transmission amplitude and the number of branch points ($R^2 = 0.0006$, $p > 0.9$), tuft surface area ($R^2 = 0.059$, $p > 0.3$) or convex Hull surface area ($R^2 = 0.0032$, $p > 0.7$; Figure 4.6A and 4.6B1-3). Importantly there was no difference between the mean number of branch points and membrane surface area of the eighteen reconstructed pairs for this purpose and the morphological data set obtained from individual, non - paired mitral cells analyzed in chapter 3 (Figure 4.6C; $p > 0.05$; Pimentel & Margrie, 2008).

Altogether the data presented above suggests that although the anatomical locus of lateral transmission is likely to be within the glomerulus, the observed variability

in its incidence, directionality and amplitude is not the result of damage to dendritic tufts during the slicing procedure nor due to naturally occurring morphological differences.

Discussion:

Altogether these data suggest that all mitral cells sharing the same glomerulus are interconnected via gap junctions with their tufts forming a large "reticulum-like" ensemble of dendrites. Whenever two mitral cells were electrically coupled this was always a reciprocal connection and it was never encountered that two cells which were confirmed to share the same glomerulus lacked electrical coupling.

Gap junctions mediate electrical coupling, between interconnected cells, via membrane pores that allow the flux of ions and even metabolites. Ionic flow is linear, bidirectional and can be of either polarity. Thus electrical coupling is a somewhat more "promiscuous" form of communication when compared to chemical transmission which, due to being dependent on the pre and postsynaptic machinery that transduces the signal, commits either to excitation or inhibition. Consistent with this notion I have observed the transfer of afterhyperpolarizing potential that follows the "presynaptic" APs. This was rarely observed in situations in which excitatory input was not blocked, suggesting that this might often be masked by excitatory inputs to the presynaptic cell such as self excitation or even direct chemical transmission from other sources. In a scenario in which both excitatory and inhibitory transmission are enabled the postsynaptic cell should be sensitive to the net result of the balance of inhibitory and excitatory conductances. It is nevertheless possible that depending on the relative locations of AHP channels to the gap junctions these might have different contributions to the overall transferred potential when compared to synaptic input. For instance self excitation which is located near the tuft might be preferably transferred when compared to the AHP if the conductances that underlie it were to be located at the soma. Given that

dendrotomized mitral cells (chapter 3; figure 3.4B) do have AHPs these conductances are not exclusively located at the tuft but nevertheless remains unclear if a fraction of AHP channels reside in the tuft. It is thus possible that self excitation and the AHP might "compete" for transference having canceling effects on the net ion flow across the gap junction.

Lateral transmission between mitral cells, until now had been proposed to rely solely on gap junction-mediated transfer of self excitation (Schoppa & Westbrook, 2002; Christie *et al.*, 2005). The direct evidence for this suggestion comes from the observed lack of lateral transmission in the connexin36 knockout mouse (Schoppa & Westbrook, 2002; Christie *et al.*, 2005; Christie & Westbrook, 2006) and is supported by the lack of anatomical evidence for the existence of chemical synapses between mitral cells. My studies searched for a correlation between self excitation, electrical coupling and lateral excitation. Having confirmed the ubiquitous expression of self excitation and electrical coupling in mitral cells it was surprising to find that lateral transmission did not follow such reliability. Despite being observed in most potential connections, the number of connections in which it was not present was considerable. In addition its amplitude correlated neither with the strength of electrical coupling alone nor when it was combined with self excitation amplitude. For the same pair some connections showed small or undetectable lateral transmission while others had strong lateral transmission even if with modest self excitation (Pimentel & Margrie, 2008).

All together these data suggest that electrical coupling cannot solely account for the degree of lateral transmission. While there must be a contribution of electrical coupling to lateral transmission it seems very unlikely that this is the sole "player" and in addition, as discussed before depending on the circumstances it might contribute with net inhibition rather than excitation. This strongly hints towards the existence of an independent form of communication between mitral cells which ultimately depends on a form of chemical transmission rather than being due to

passive electrical coupling. However it is possible that mitral cells sharing the same glomerulus might always be chemically coupled and the apparent lack of lateral excitation may be due to dendritic filtering within the tuft and apical dendrite of comparatively smaller or more distant lateral excitation (Urban & Sakmann, 2002). However previous studies have suggested that tufts are spatially uniform and likely function as a single compartment and that subthreshold EPSP attenuation in the apical dendrite is weak when compared to other neuronal types and thus less likely contribute to such phenomena (Djurisic *et al.*, 2004; Djurisic *et al.*, 2008).

Given that this form of communication is a phenomenon exclusive to intraglomerular cells the most obvious locus should be the glomerulus. Another possibility would be that the lateral dendrites from cells that project to the same glomerulus might more closely appose in order to facilitate chemical transmission. In order to investigate this possibility I have analyzed the minimum dendritic distance between apical tuft dendrites and the lateral dendrites. Consistently the closest distance observed between lateral dendrites was substantially larger than in the tuft and thus much less likely to provide the proximity required for direct chemical transmission. Further support for a glomerular locus for lateral excitation comes from recordings performed along the apical dendrite while evoking action potentials in the presynaptic mitral cell (Urban & Sakmann, 2002). They demonstrated that single action potential evoked lateral excitation events occur earlier and are larger in the distal apical dendrite when compared to those recorded at the soma. The anatomical data presented here further corroborates this finding in that I do not observe lateral excitation when the apical tuft of one mitral cell has been amputated but the cell is otherwise in close proximity to its intraglomerular partner. In addition, lateral excitation occurs in pairs for which the lateral dendrites do not closely oppose one another and thus would be unable to mediate chemical transmission. Altogether it is thus likely that chemical lateral excitation mediated via AMPA receptors is exclusively glomerulus-based. The morphometric analysis of mitral cell

pairs indicates that macroscopic features of tuft anatomy do not explain the likelihood of lateral excitation between a given cell pair (Pimentel & Margrie, 2008).

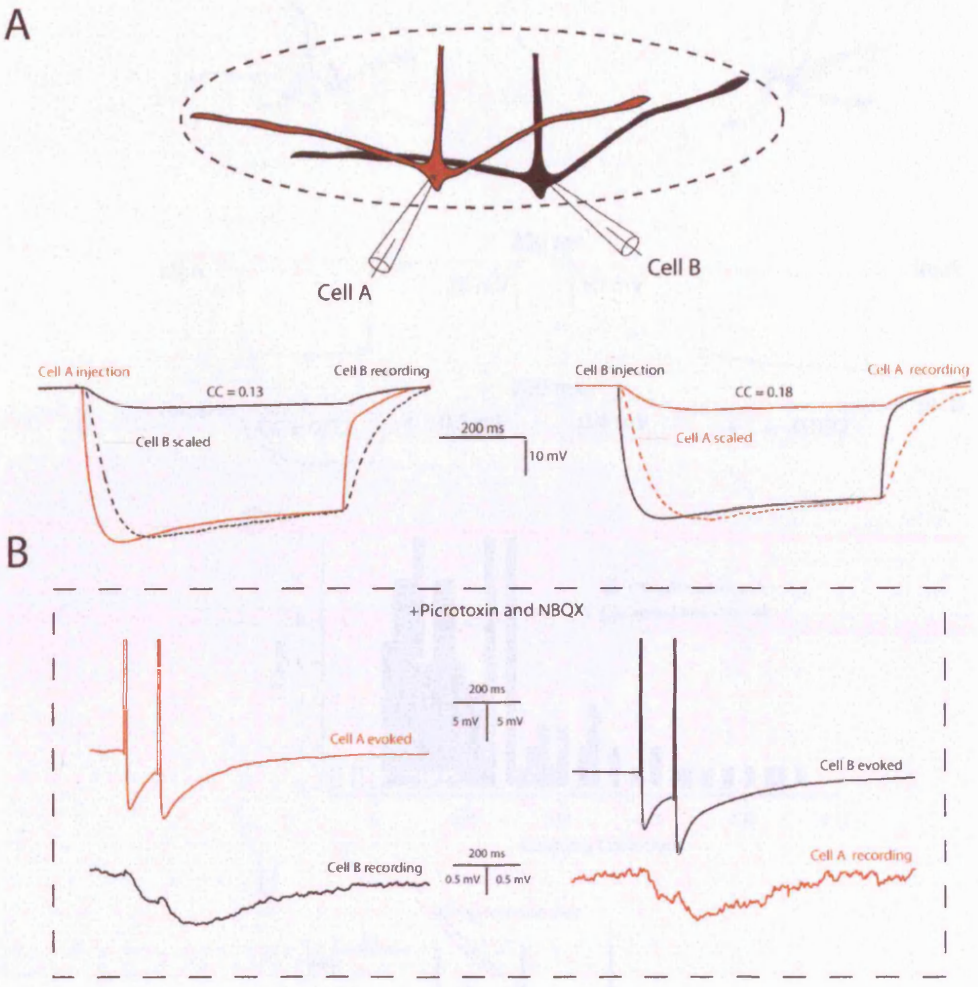

Figure 4.1 - Electrical coupling between mitral cells
A - Electrical coupling can be assessed by sequential injection of hyperpolarizing pulses in each cell while simultaneously recording from two mitral cells. Example average traces (30 sweeps) showing bi-directional electrical coupling. Doted lines represent the electrically transferred (recorded) hyperpolarization to the postsynaptic cell which has been scaled according to the respective coupling coefficient (CC) of the connection.
B - Occasionally blocking of inhibitory and excitatory synaptic input by picrotoxin and NBQX respectively uncovered the coupling of the AHP.

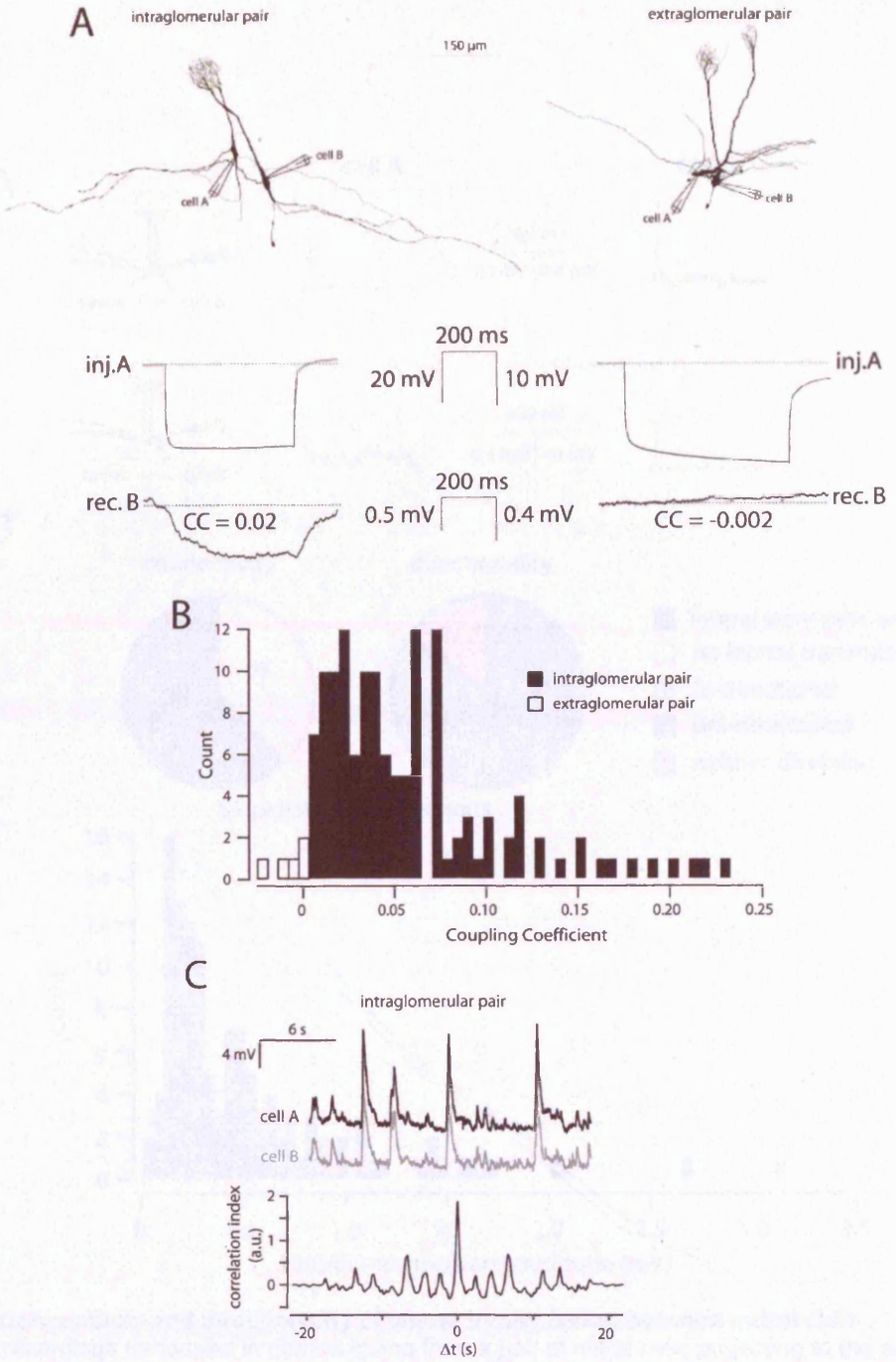

Figure 4.2 - Electrical coupling is exclusive and ubiquitous to intraglomerular mitral cells
A - (Top) Example reconstructions of pairs of mitral cells tested for electrical coupling that belonged to the same glomerulus (intraglomerular; left) and to different glomeruli (extraglomerular; right). (Bottom) example average traces of the pulses used to access electrical coupling (only one direction shown) for the examples shown above. Intraglomerular pairs always showed electrical coupling while extraglomerular pairs never did.
B - Histogram showing the distribution of coupling coefficients for 133 connections from intraglomerular pairs (filled bars) and 5 extraglomerular pairs (non-pairs; open bars).
C - (Top) Example traces of simultaneous recording of spontaneous activity in an intraglomerular pair of Mitral cells. Cross-correlation of the two traces reveals that subthreshold membrane voltage fluctuations LLD's are highly synchronous (bottom).

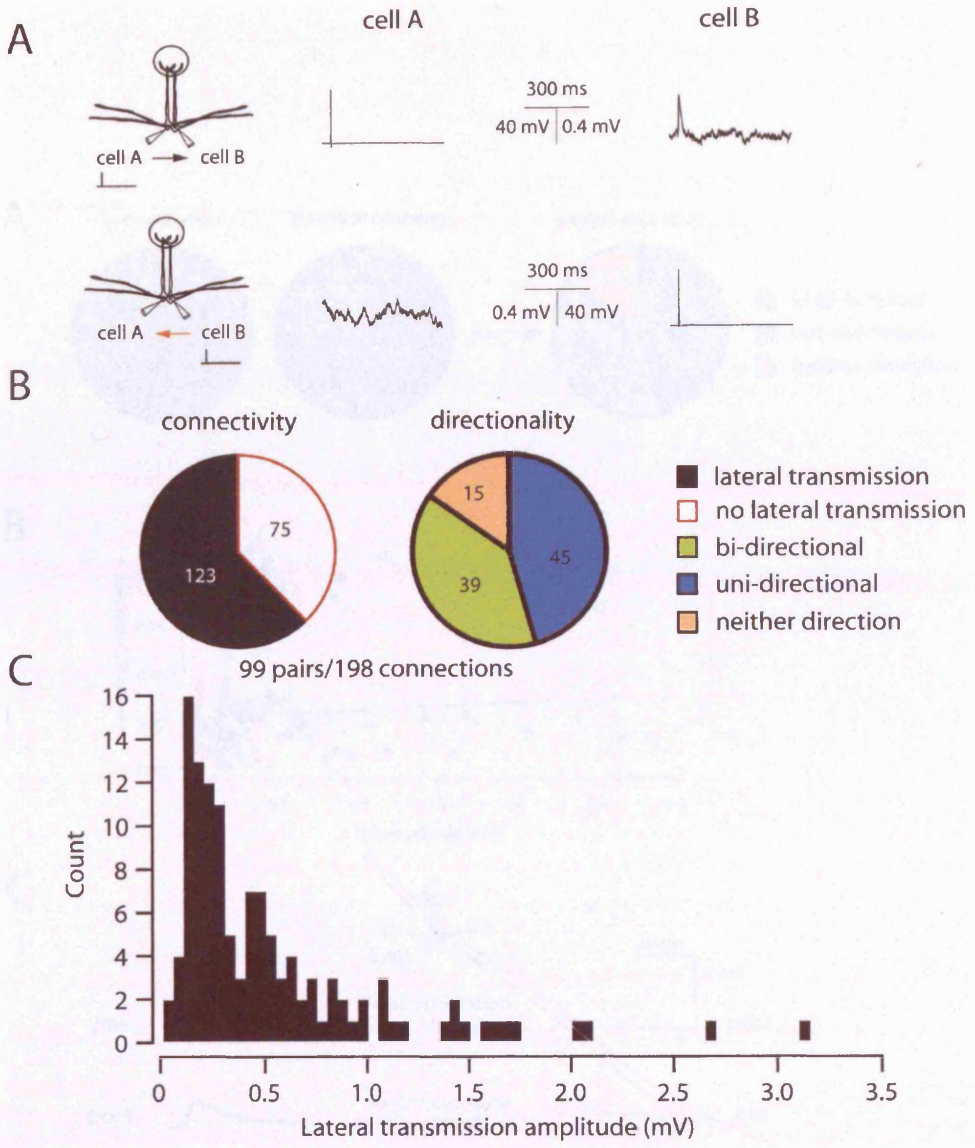

Figure 4.3 - Connectivity and directionality of lateral transmission between mitral cells
A - Example recordings performed in current clamp from a pair of mitral cells projecting to the same glomerulus. Evoking a single AP in one cell (top left) can result in a detectable depolarization in the other cell (top right). This form of lateral transmission was not always bi-directional (bottom).
B - Pie charts showing the overall connectivity and directionality of lateral transmission across 99 pairs of mitral cells (198 possible connections).
C - Histogram showing the distribution of lateral transmission amplitude for 123 connections from intraglomerular pairs.

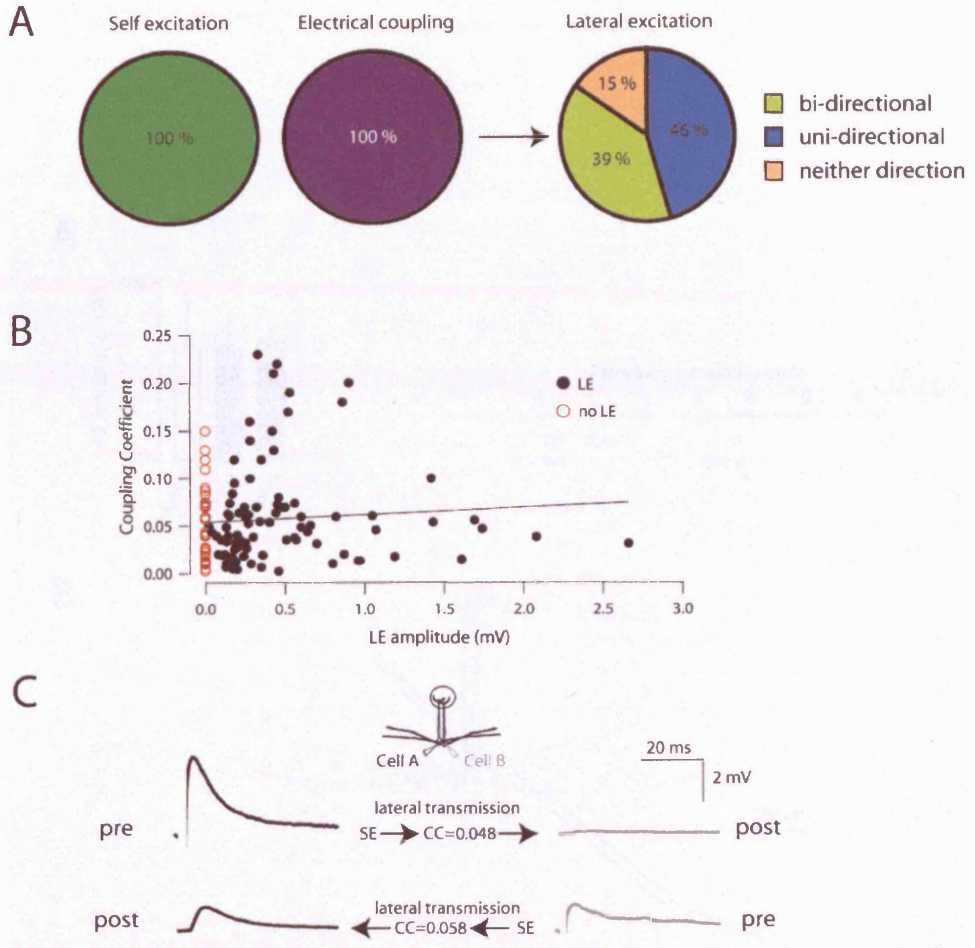

Figure 4.4 – Lateral transmission amplitude does not correlate with electrical coupling and self excitation

A - Lateral excitation reliability and connectivity does not follow that of self excitation and electrical coupling.
B - Plot showing the lack of correlation between electrical coupling (CC) and lateral transmission amplitude.
C - Example traces (15 sweeps) from a simultaneously recorded pair of cells in which SE, coupling coefficients and LE were determined. In one direction (cell A→B) large self excitation (3.60 mV) was observed that resulted modest lateral transmission (0.15 mV). In contrast in the other direction (cell B→A) I observed (1.05 mV) lateral transmission that was similar amplitude to self excitation in cell B (1.13 mV). Those connections (from the same pair) showed a very similar degree of electrical coupling.

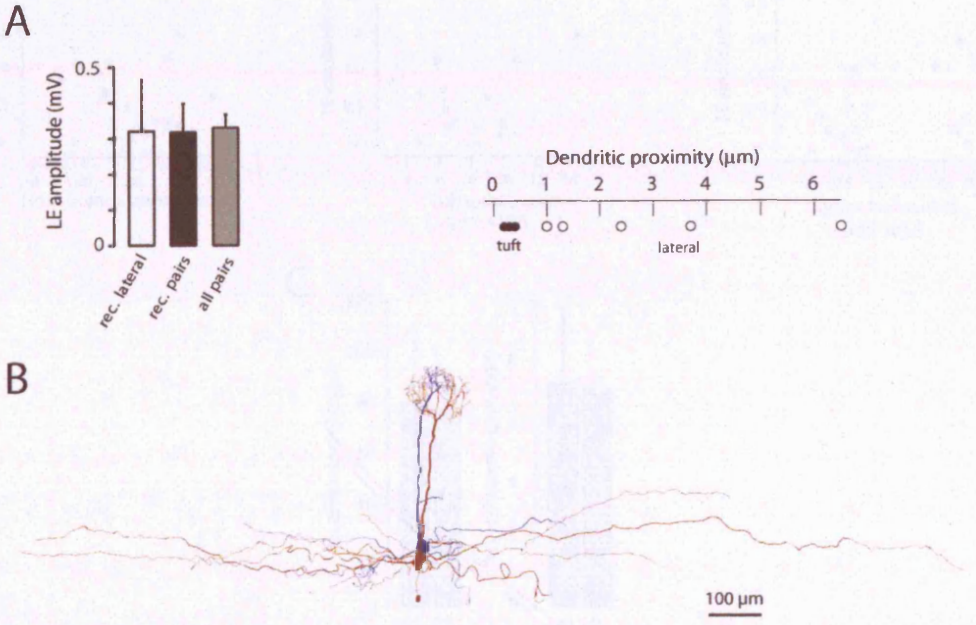

Figure 4.5 – Lateral transmission is unlikely to occur via the lateral dendrites
A - (Top left) A plot showing the amplitude of lateral transmission for pairs where lateral dendrites were reconstructed (rec. laterals, n = 5 pairs), all pairs where only tufts were reconstructed (rec. pairs n = 18 pairs) and for the entire data set (all pairs, n = 99 pairs; bars correspond to standard error). (Top right) Comparison of the minimum distance bin (bins were 0.1 µm wide; lower bin limit plotted - see Methods) obtained from a histogram of dendritic distances generated using Neurolucida Explorer (filled circles apical tuft, blank circles lateral dendrites) from 5 reconstructed pairs one of which is provided below (4.5B).
B - Example reconstruction of one of the quantified pairs.

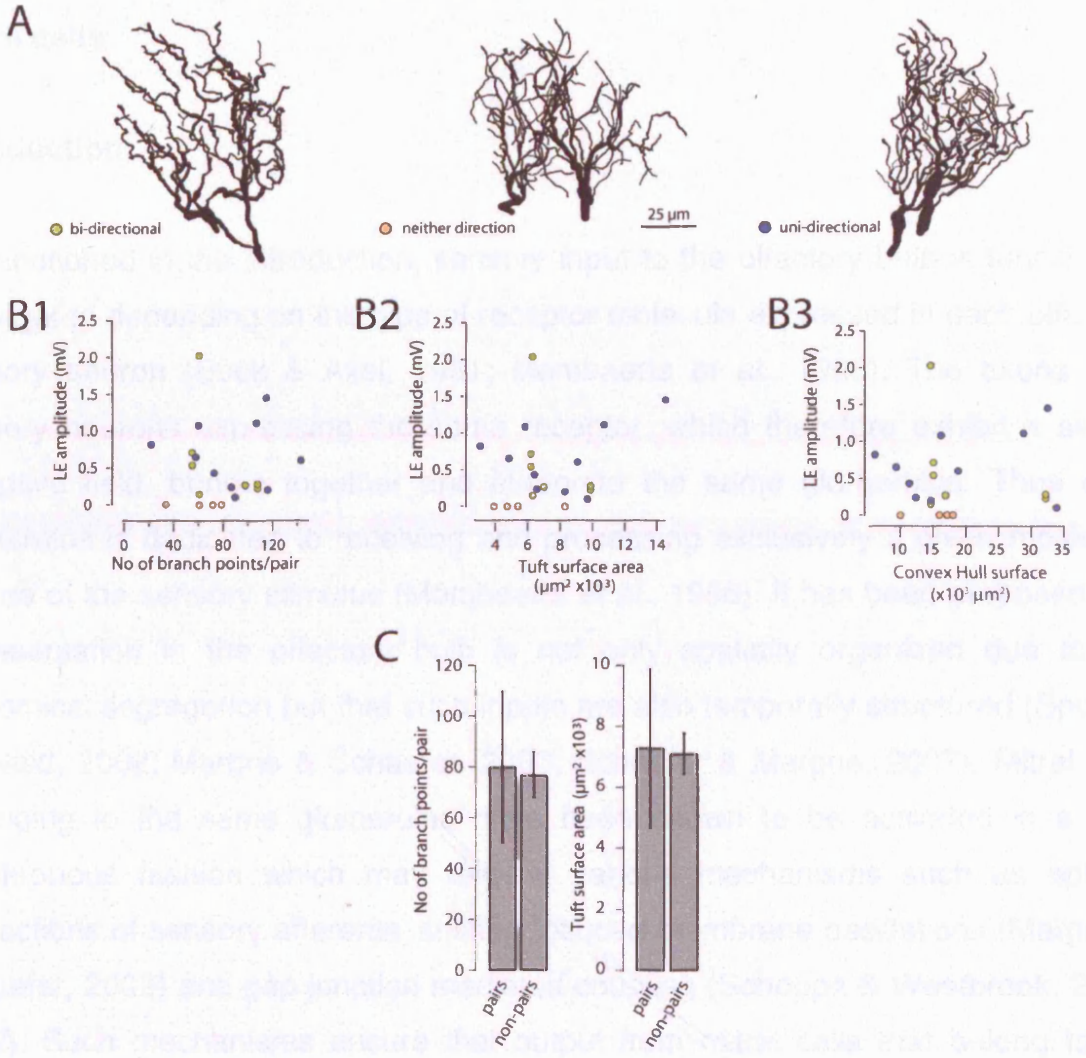

Figure 4.6 – The efficacy and occurrence of lateral transmission do not correlate with tuft morphology
A - Example Neurolucida reconstructions of pairs of dendritic tufts belonging to each of the three lateral transmission directional groupings.
B - Graphs showing the relationship between the total number of branch points (B1), surface area (B2) and convex Hull surface (B3) and the amplitude of lateral transmission (bi-directional (both directions plotted) n = 8 connections (green circles); uni-directional (a single direction plotted) n = 10 (blue circles); neither direction (a single direction plotted) of n = 4 (red circles). (Total n = 22 connections from 18 pairs).
C - Comparison of the average morphometric properties of both reconstructed tufts per pair (n = 18 pairs) to those from individual cells that did not project their apical dendrites to the same glomerulus (non-pairs; n = 12 cells, each tuft multiplied by a factor of 2; error bars correspond to standard deviation).

Chapter 5: Chemical transmission and plasticity between intraglomerular mitral cells

Introduction:

As mentioned in the introduction, sensory input to the olfactory bulb is functionally segregated depending on the type of receptor molecule expressed in each olfactory sensory neuron (Buck & Axel, 1991; Mombaerts *et al.*, 1996). The axons from sensory neurons expressing the same receptor, which therefore exhibit a similar receptive field, bundle together and innervate the same glomerulus. Thus each glomerulus is dedicated to receiving and processing exclusively a given molecular feature of the sensory stimulus (Mombaerts *et al.*, 1996). It has been proposed that representation in the olfactory bulb is not only spatially organized due to the anatomical segregation but that such inputs are also temporally structured (Spors & Grinvald, 2002; Margrie & Schaefer, 2003; Schaefer & Margrie, 2007). Mitral cells belonging to the same glomerulus have been shown to be activated in a very synchronous fashion which may rely on various mechanisms such as ephatic interactions of sensory afferents, sniffing induced membrane oscillations (Margrie & Schaefer, 2003) and gap junction mediated coupling (Schoppa & Westbrook, 2001, 2002). Such mechanisms ensure that output from mitral cells that belong to the same functional assembly likely provide similar output patterns to different target cell populations in downstream structures (Suzuki & Bekkers, 2006)

Despite extensive studies on how recurrent and lateral (feed-forward) inhibition might be involved in the shaping of glomerular activity it is unclear whether similar excitatory links exist within or between the principal cells of different glomeruli (Pinching & Powell, 1971a; Jahr & Nicoll, 1980; Aungst *et al.*, 2003; Murphy *et al.*, 2005). Mitral cells are known to express recurrent-excitation (self-) and

simultaneous recordings have also provided evidence of an intraglomerular excitatory link (lateral excitation; Schoppa & Westbrook, 2002; Urban & Sakmann, 2002; Christie & Westbrook, 2006). Anatomical evidence for a direct form of chemical communication between mitral cells is lacking and therefore lateral excitation between mitral cells has been proposed to be a consequence of the electrical transfer of recurrent excitatory potentials via gap junctions. Data discussed in the previous chapters strongly suggests that these forms of communication might be independent from each other and that possibly there is a direct chemical link between mitral cells. This would provide mitral cells with a specific mechanism by which each cell could influence the excitability of its intraglomerular partners. Chemical lateral excitation, would be exclusively excitatory and provided that the necessary conditions for release to occur are met it will contribute exclusively to the depolarization of the intraglomerular partner and thus contribute to action potential discharge. Determining how the discharge of an individual mitral cell directly impacts on the excitability of the glomerular mitral cell assembly, will therefore enhance our understanding of the mechanisms of sensory representation within and across such functional modules.

Glutamatergic transmission at axo-dendritic synapses has been shown to provide a locus for plasticity (Malenka & Bear, 2004). In the olfactory bulb plasticity within dendro-dendritic networks has long been proposed to provide a mechanism for olfactory memory, (Rosser & Keverne, 1985; Kaba & Keverne, 1988; Sullivan *et al.*, 2000) sensory adaptation (Li, 1990), tuning receptive fields and even discrimination learning (Wilson *et al.*, 2004). Despite these proposed benefits, evidence for neuronal plasticity between dendritic connections in the bulb or for dendritic transmission in general has not been forthcoming. This raises questions as to whether or not dendritic transmission is functionally more hard-wired than axonal transmission and whether or not dendro-dendritic transmission in the olfactory bulb can provide the kind of activity dependent plasticity observed at many axo-dendritic synapses. In this chapter I further explore the possibility that lateral excitation is an

independent form of chemical communication and investigate whether it could provide the olfactory bulb with a locus for long term plasticity.

Results:

Pharmacological profile of lateral transmission

The previous chapters have provided evidence that lateral transmission, at least in part, is an independent form of communication largely independent of electrical coupling and self excitation. The observed connectivity, directionally and even amplitude of lateral transmission contrast highly to the ubiquitous and reliable expression of self excitation and electrical coupling. The possibility of a direct form of chemical communication between mitral cells is very interesting as it could provide an exclusively excitatory mechanism by which mitral cells in the same network influence their partner's excitability. Given the finding that self-excitation is mediated largely by CP-AMPA receptors and considering that calcium impermeable AMPA receptors also exist in the olfactory bulb I pursued the possibility that self and lateral transmission might rely on different subtypes of AMPA receptors. In contrast to self excitation, I have found that lateral transmission was largely insensitive to the CP-AMPA receptor specific antagonist NAS (80.8 ± 21.4% of control amplitude $n = 7$, $p > 0.05$ Wilcoxon paired sample test; Figure 5.1B and C) but was abolished by the general AMPA receptor antagonist NBQX (residual was 15.8 ± 0.1%; figure 5.1B and C). It therefore seems that this form of mitral cell – mitral cell communication is mediated by calcium impermeable receptors. Given that in chapter 3 I showed that self excitation is mediated by CP-AMPA receptors, these experiments confirm that self excitation (within) and lateral transmission (between mitral cells) are predominantly independent forms of AMPA receptor mediated excitation (Figure 5.1D, Mann-Whitney $p < 0.05$) which might play independent roles in glomerular processing (Pimentel & Margrie, 2008).

Short-term dynamics of lateral transmission

The uncovering of lateral transmission as an independent form of chemical transmission meant that its properties as a chemical synapse are yet uncharacterized. Therefore I profiled its short term dynamics as this could provide clues to the integrative properties of this mode of communication in mitral cells. As for self excitation (please see chapter 3) I evoked pairs of APs at various frequencies but this time, APs were induced on a presynaptic mitral cell while recording the evoked responses in the connected mitral cell. Example traces for such an experiment are shown in Figure 5.2A (A1-A5 corresponding to 100, 50, 20, 10 and 5Hz respectively). In order to assess the impact of frequency on the amplitude of the second event the ratio of the two, the paired pulse ratio (PPR = 2^{nd} PSP amplitude / 1^{st} PSP amplitude), was determined after subtracting summation induced by the first EPSP. Figure 5.2B illustrates the population short term dynamics profile for lateral excitation. I found that the range of PPR ratios was typically greater for lateral excitation than that for self excitation. For a given frequency self excitation showed a trend of either facilitation or depression while for lateral excitation generally a mixture of both was observed (Figures 3.4B and 5.3A). Such differences between the short term dynamics profiles of self excitation and lateral excitation were more striking for higher frequencies (Figure 5.3 A and B). At 100Hz the mean PPR for lateral excitation was significantly different from that observed for self excitation (figure 5.3B; $p < 0.01$ Mann-Whitney). Furthermore the cumulative distribution of the population for lateral excitation and self excitation were significantly different (Kruskal-Wallis test $p < 0.05$) which adds to the notion that self and lateral excitation are independent forms of chemical communication serving independent roles in olfactory processing.

Lateral excitation plasticity

As mentioned before chemical synapses have been known to exhibit long term forms of plasticity, however direct evidence for such forms of plasticity in olfactory bulb synapses have not been forthcoming. I therefore investigated whether physiologically relevant patterns of activity in mitral cells could modulate lateral excitation between mitral cells.

During the active process of odour sampling (Kay & Laurent, 1999) and even during odour expectation (Uchida & Mainen, 2003; Kepecs *et al.*, 2006), it has been shown that sniff behaviour in rodents can alter. This indicates that each sniff might be involved in facilitating odour processing (Kepecs *et al.*, 2006; Schaefer *et al.*, 2006; Schaefer & Margrie, 2007). *In vivo,* it was observed that mitral cell activity is locked lock to the sniff cycle. This occurs in the theta frequency range (sniffing frequency ~5 Hz) within which mitral cells generally fire action potentials in short bursts at approximately 50-60 Hz (Margrie & Schaefer, 2003). I therefore explored the possibility that lateral excitation might be sensitive to similar temporally-patterned inputs (Pimentel & Margrie, 2008). In order to do so I used a theta-burst stimulation (TBS) protocol designed to approximate the activity of mitral cells observed *in vivo* during sniffing (see methods for detailed description). An example of such a burst can be found in Figure 5.4A. I found that TBS had different effects in separate subgroups of connections. While I found that it induced potentiation of lateral excitation at some, in others lateral excitation became depressed (examples in Figure 5.4B1 and B2). Thus the same stimulation protocol had a different impact on the amount and direction of change of lateral excitation efficacy (figure 5.5A). Plotting the percentage amplitude change against the initial lateral excitation amplitude revealed a linear correlation in which weaker connections exhibited potentiation while larger ones underwent depression ($R^2 = 0.53$, $p > 0.01$; $n = 18$; Figure 5.5B; Pimentel & Margrie, 2008).

As mentioned before long term changes in synaptic efficacy can be the reflection of presynaptic or postsynaptic changes or even a combination of both. In order to investigate the locus of the observed lateral excitation plasticity in a subset of cells I first used a paired pulse protocol given that it can provide an assay for potential changes in the probability of transmitter release (Fatt & Katz, 1952; Zucker & Regehr, 2002). In the examined connections TBS- induced changes in lateral excitation strength expressed changes in the paired pulse ratio (R^2 =0.91, $p < 0.05$, Figure 5.6A n = 5/5). An Increase in lateral excitation efficacy was associated with a decrease in the paired pulse ratio and vice versa which would be consistent with a change in release probability. To further explore this possibility I determined whether the changes in the direction and amplitude of plasticity were accompanied and consistent with changes in the coefficient of variation of lateral excitation. Normalized CV^2/amplitude plots are often used as an indication of the locus of changes in synaptic transmission whereby gradients steeper than unity are considered predominantly presynaptic (Malinow & Tsien, 1990; Faber & Korn, 1991; Hardingham *et al.*, 2007). Overall I found that on average the gradient of the ratiometric change in the coefficient of variation for both depressed and potentiated connections is steeper than the unity line revealing that a presynaptic component might be involved (Figure 5.6B1 and 5.6B2 respectively). Even in the instances in which connections fell near or outside the predicted presynaptic quadrant (see Figure 5.6C1), they typically displayed large changes in lateral excitation variance in the predicted direction (for example see Figure 5.4B1-2). However, in such cases, it appeared that the observed change in the amplitude of the EPSP occured to a lesser extent than expected for a purely presynaptic expression (example Figure 5.4B1-2, 5.6C1-2) which could be due to a counteracting postsynaptic mechanism. Nevertheless CV^2 analysis together with the paired pulse data suggests that a presynaptic mechanism is likely involved in lateral excitation plasticity within intraglomerular mitral cells (Pimentel & Margrie, 2008).

Discussion:

Previous studies had suggested that lateral excitation between mitral cells in the olfactory bulb is an epiphenomenon of the existence of self excitation and gap junction mediated electrical coupling (Schoppa & Westbrook, 2001, 2002; Christie *et al.*, 2005; Christie & Westbrook, 2006), rather than an independent form of chemical communication. Such hypothesis was supported by the lack of anatomical evidence to support a direct synaptic connection between mitral cells (Price & Powell, 1970; Pinching & Powell, 1971b; Kosaka & Kosaka, 2004, 2005b) and more directly by the fact that lateral excitation is disrupted within the Connexin36 KO background. The observation that self excitation and lateral excitation rely on the activation of different types of AMPA receptors provides direct evidence for the existence of an independent channel of chemical communication. While self excitation is mediated by calcium-permeable AMPA receptors, lateral excitation shows little sensitivity to a specific antagonist (NAS) for these receptors. This suggests that they might serve independent roles in olfactory processing and perhaps be independently regulated. As mentioned before, calcium-permeable AMPA receptors have been shown to be the mediators of some forms of synaptic plasticity (Cull-Candy *et al.*, 2006) and in the retina, where expressed presynaptically, shown to be the source of calcium directly involved in release of neurotransmitter (Chavez *et al.*, 2006). The finding that lateral excitation is largely insensitive to NAS suggests that calcium-permeable AMPA receptors are involved neither in lateral excitation release or reception within intra-glomerular mitral cell dendrites.

Previous studies had suggested that lateral transmission between mitral cells relied solely on gap junction-mediated transfer of self excitation (Schoppa & Westbrook, 2002; Christie *et al.*, 2005). The direct evidence to support this claim came from a lack of lateral transmission in the connexin36 knockout background (Schoppa & Westbrook, 2002; Christie *et al.*, 2005; Christie & Westbrook, 2006). In addition

chemical transmission between mitral cell pairs in the Cx36 KO background was only observed during periods of high frequency trains of activity while in the presence of glutamate uptake blockers which artificially increases glutamate concentration (Christie & Westbrook, 2006). In contrast I found that under more physiological conditions lateral excitation can be evoked by a single action potential without the need of inducing artificially high concentrations of glutamate. Therefore it is likely that lateral excitation contributes to intra-glomerular processing upon moderate mitral cell activation. In addition the short term dynamic profiles of self and lateral excitation are markedly different. The range of paired pulse ratios is larger for lateral excitation and for the same frequencies both facilitation and depression were observed consistent with a form of communication that can express changes in release probability such as synaptic transmission. Altogether these data suggest that while gap junction-mediated coupling may be a requirement for the specification of chemical lateral excitation, the degree of coupling cannot exclusively determine the efficacy of lateral excitation (Pimentel & Margrie, 2008).

It has been well accepted that dendritic release can be modulated either by the action of neuromodulators (Isaacson & Vitten, 2003; Davison *et al.*, 2004) or intense bursts of activity (Wilson & Nicoll, 2002; Freund *et al.*, 2003). Here I show that in mitral cells, bursts of action potentials in the theta frequency, which mimic the *in-vivo* patterns of activity for these cells, can regulate the strength of intra-glomerular dendritic transmission. The sensitivity of lateral excitation to such patterns of activity suggests that its efficacy will likely be modulated under physiological conditions *in vivo* (Margrie & Schaefer, 2003; Schaefer *et al.*, 2006; Schaefer & Margrie, 2007; Verhagen *et al.*, 2007). In olfactory bulb slices, theta-burst stimulation could induce bidirectional changes in lateral excitation efficacy such that small connections were potentiated while larger connections showed depression (Pimentel & Margrie, 2008). Recent work in layer 2/3 neocortical pyramidal cells reported similar relationship between the initial strength of axo-dendritic connections in determining the direction and the amount of change in efficacy by a mechanism that is

dependent on changes of release probability (Hardingham *et al.*, 2007). Consistent with this study, I observed that the direction and amplitude of lateral excitation plasticity correlates with changes in the paired pulse ratio. As with layer 2/3 neurons (Hardingham *et al.*, 2007), changes in the CV^2 before and after induction of plasticity further indicate a presynaptic contribution (Pimentel & Margrie, 2008).

So far the major form of plasticity described within the olfactory bulb circuitry is due to neurogenesis and the integration of new interneurons into the circuitry, which occurs over the time course of several weeks (Lledo *et al.*, 2006). My data shows that more instantaneous forms of dendro-dendritic plasticity are also available to this structure. A possibility is that lateral excitation serves the role of providing a mechanism by which the gain function of an individual mitral cell can be modulated to match that of the overall glomerular input. This could compensate for flux in the ongoing degeneration and regeneration of the sensory input that each individual mitral cell receives. This way lateral excitation plasticity would normalize sensitivity to provide reliable sensory reception and thus also transmission via the intraglomerular modules. Such network homeostasis mechanisms would ensure that mitral cells sharing the same functional module provide similar output patterns to separate target cell populations in downstream structures (Suzuki & Bekkers, 2006; Pimentel & Margrie, 2008).

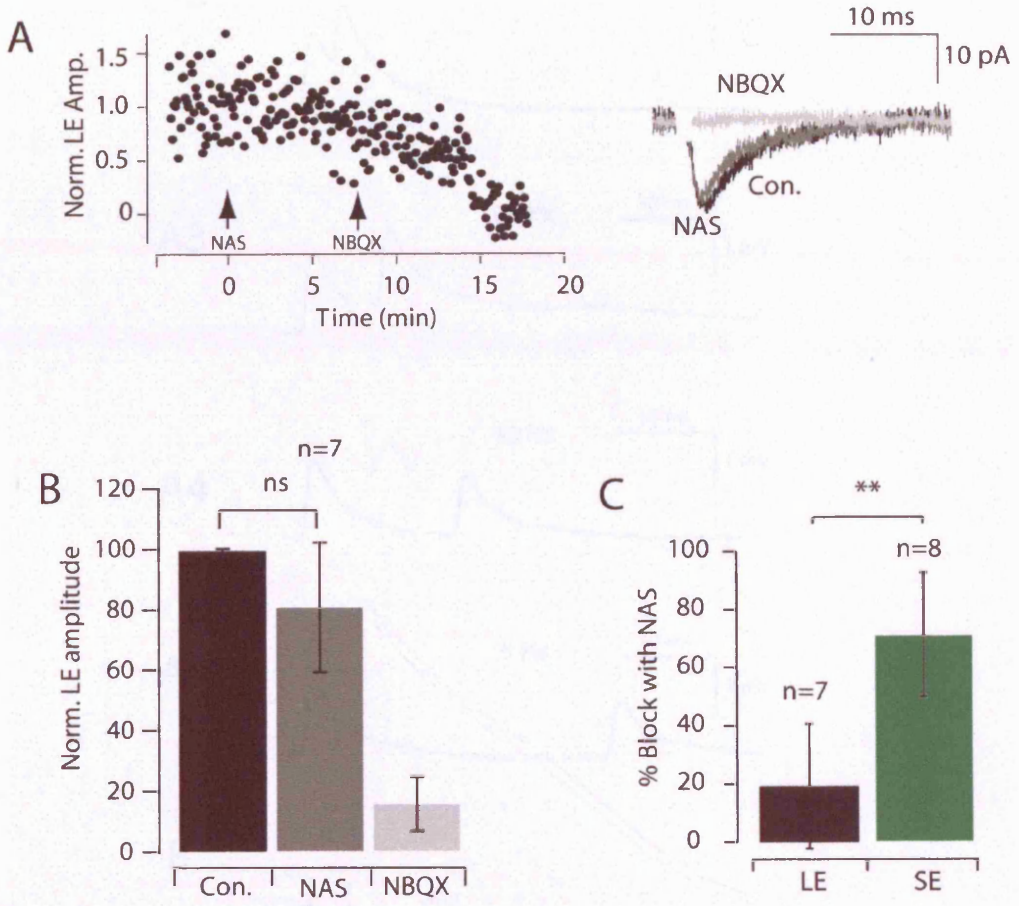

Figure 5.1 – Lateral transmission relies on calcium impermeable AMPA receptors
A - (Left) Plot of the normalized amplitude of lateral transmission over time; arrows indicate the beginning of bath application of NAS and NBQX respectively. (Right) Example traces recorded in voltage clamp (30 sweeps)
B - Bar graphs (right) for population data (n = 7) showing the lack of sensitivity of lateral transmission to NAS and block by NBQX. (bars correspond to standard deviation)
C - Lateral transmission and self-excitation show contrasting sensitivities to NAS (Mann-Whitney; $p < 0.01$; bars correspond to standard deviation)

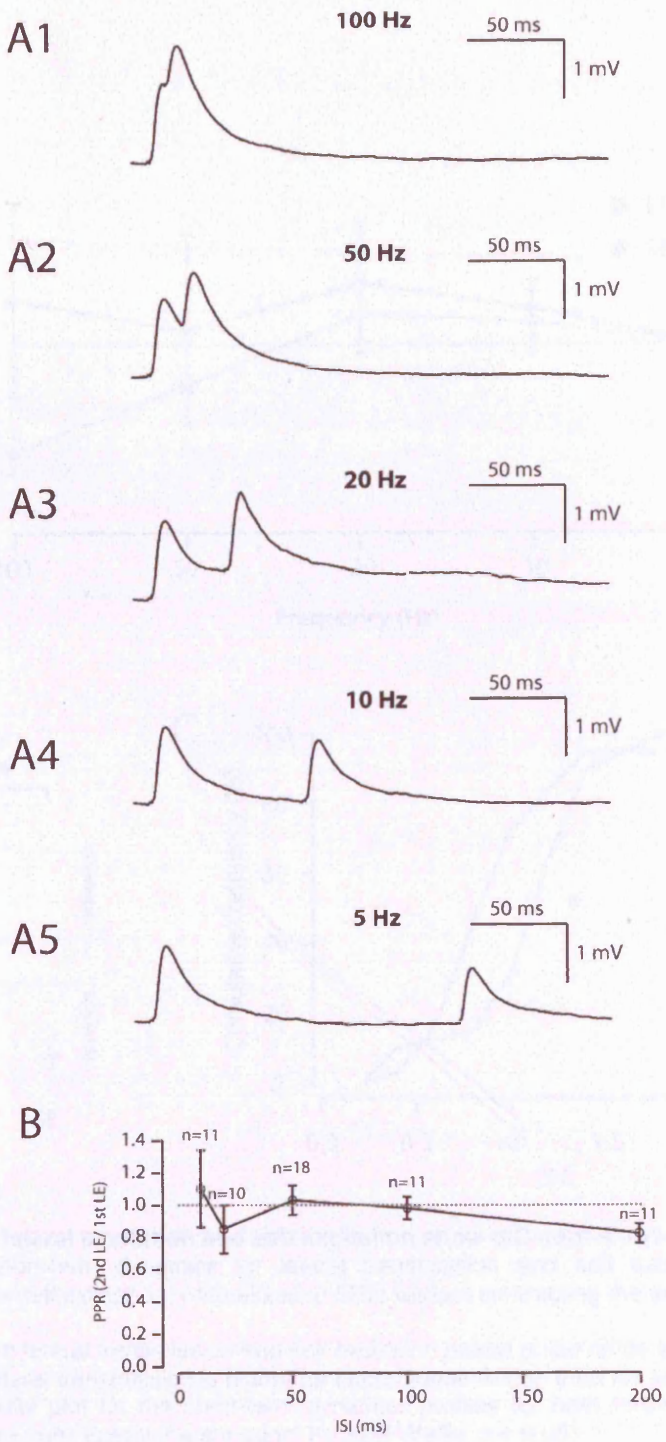

Figure 5.2 - Short term dynamics of chemical lateral excitation
A1-5 - Dependence of the inter-stimulus frequency on the short-term dynamics of lateral transmission. Two APs were evoked at varying frequencies (A1-5: 100, 50, 20, 10 and 5Hz respectively). Averaged traces (left; ~20 traces) are shown to illustrate the differential integration of lateral transmission.

B - Population data summarizing the short-term dynamics profile of lateral transmission.

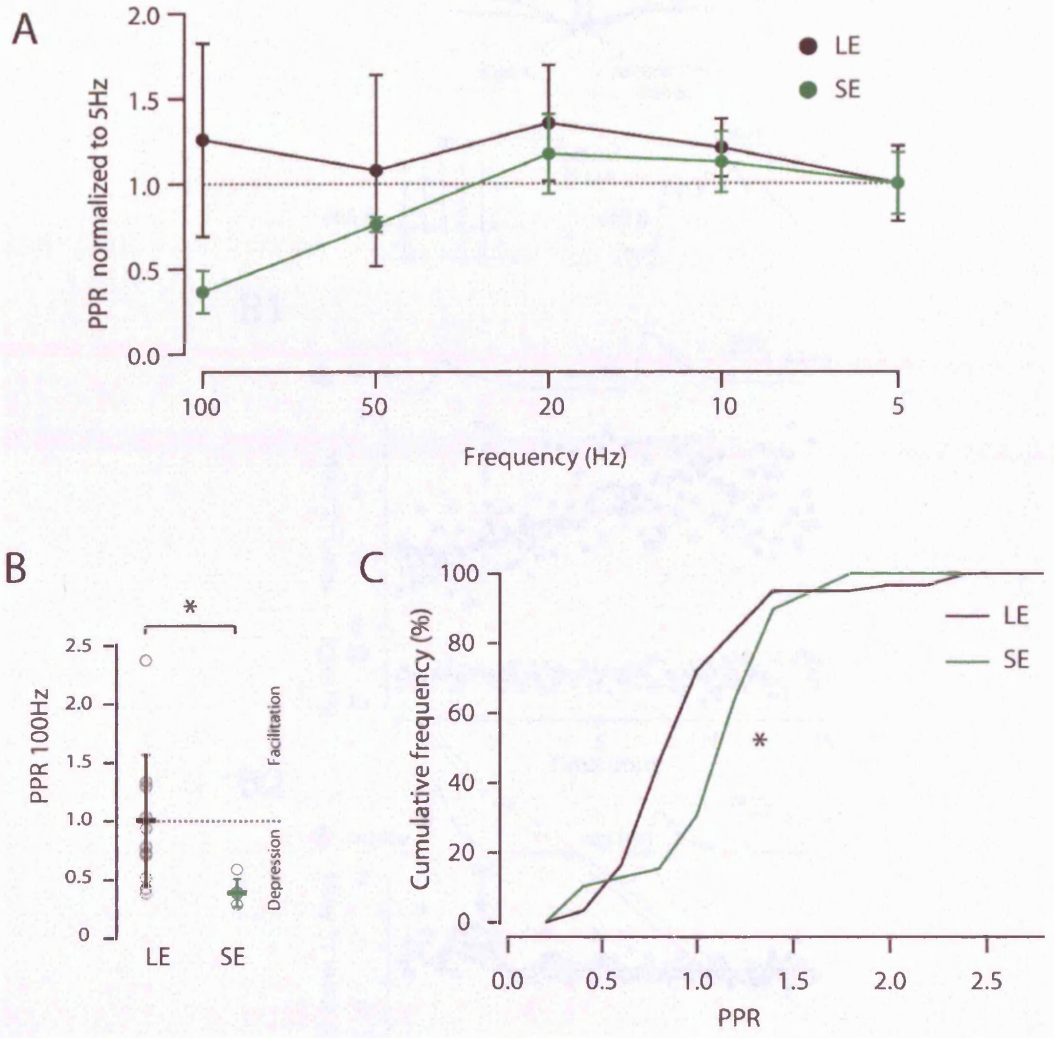

Figure 5.3 – Chemical lateral excitation and self excitation show different short-term dynamics
A - Profiles of the short-term dynamics for lateral transmission and self excitation (black dots lateral transmission, green dots self excitation; normalized to 5Hz) without subtracting the summated component of the PSPs.
B - Comparison between lateral transmission and self excitation paired pulse ratios at 100 Hz (Mann-Whitney; $p < 0.05$). Variability for lateral transmission is higher for lateral transmission than for self excitation
C - Cumulative probability plot for the short-term dynamics profiles for both forms of communication (green lines; self excitation black lines lateral transmission; Kruskal-Wallis, $p < 0.05$).

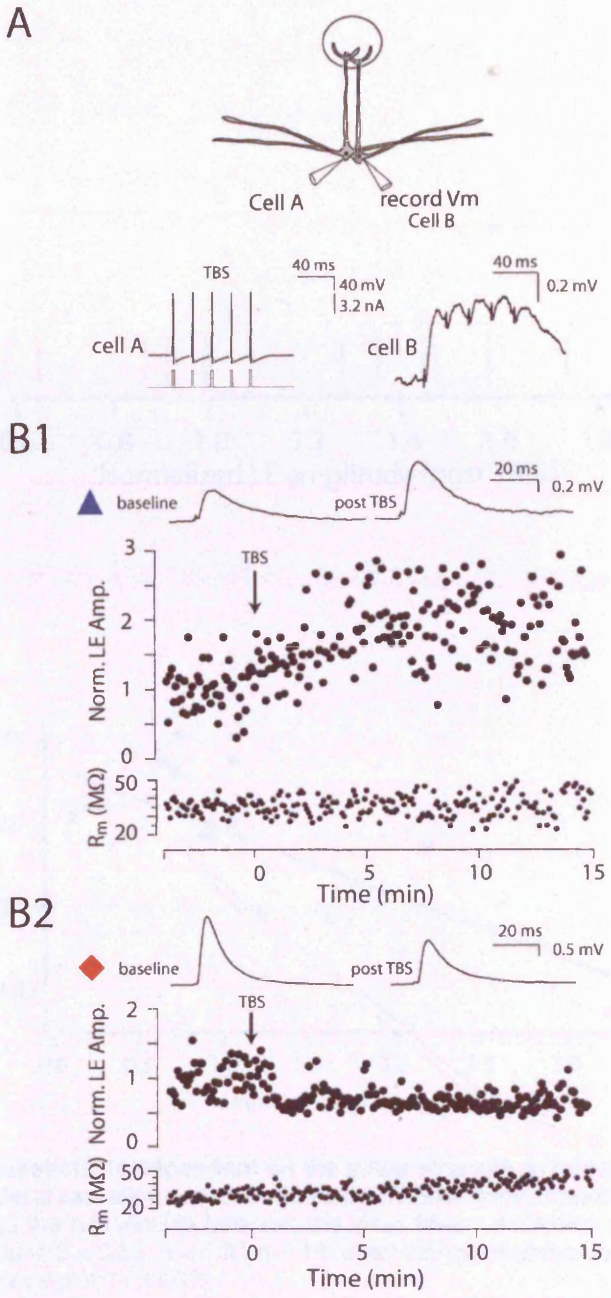

Figure 5.4 - Chemical lateral excitation exhibits bi-directional plasticity when stimulated with sniffing-like patterns of activity

A - Example of a burst of induced APs (left; 5 APs at 50Hz) and evoked lateral excitation (right) used for the theta burst stimulation (TBS) based induction protocol. TBS consisted of 150 of such bursts repeated at 5Hz (750 APs, total duration 30s).

B - Plots of lateral excitation amplitude over time illustrating examples of potentiation (B1) and depression (B2) following theta burst stimulation (onset of TBS indicated by the arrow). Average traces (top) of 30 sweeps recorded in current clamp during the baseline and following TBS. Below is a plot of input resistance measured over time using a somatic hyperpolarizing pulse.(diamond and triangle will be used to represent these examples in the population data in subsequent figures)

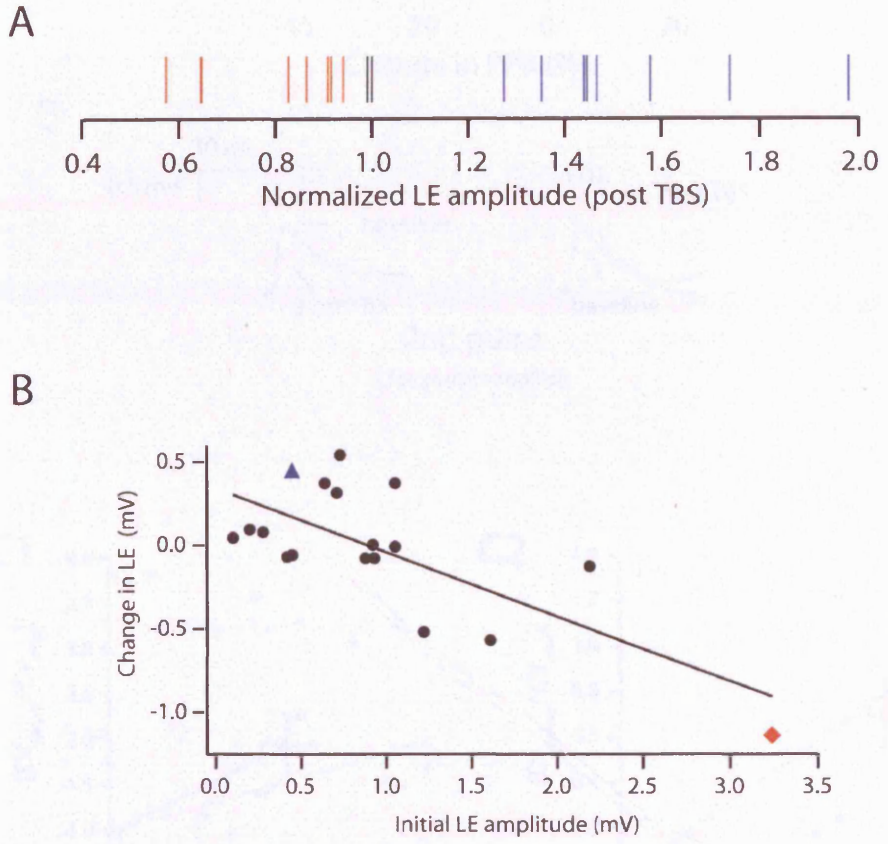

Figure 5.5 - TBS induced plasticity is dependent on the initial strength of lateral excitation
A - Normalized change in lateral excitation amplitude following TBS for each connection.
B - Population data showing the relationship between the initial lateral excitation amplitude and the observed absolute change in amplitude ($R2 = 0.53$, $p < 0.01$ $n = 18$; open triangle represents connection shown in 5.4B1, open diamond represents connection in 5.4B2).

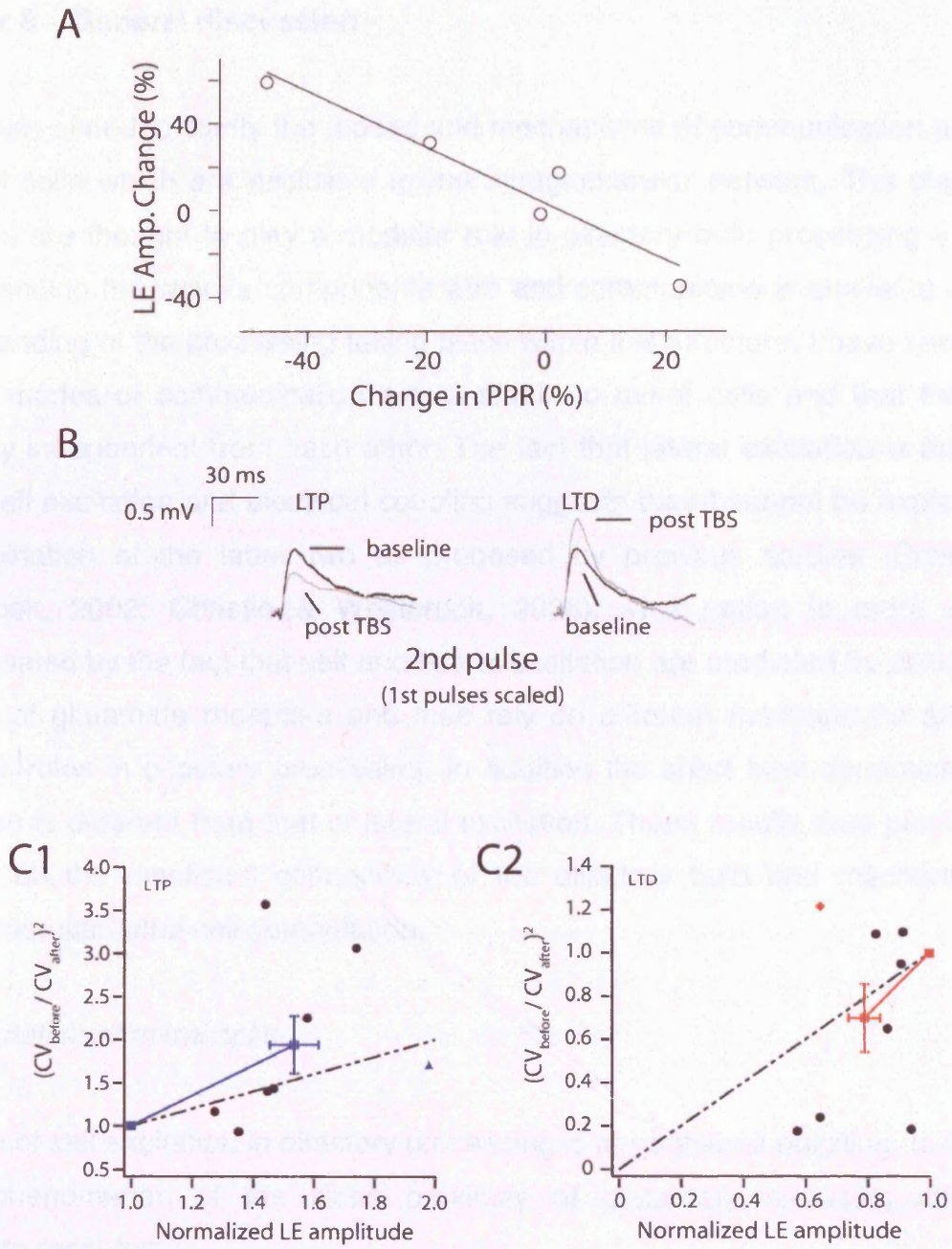

Figure 5.6 - Induction of lateral excitation plasticity is associated with a change in dendritic release
A - Plot of the change in paired pulse ratio (PPR) as it relates to changes in lateral excitation induced by TBS. Pairs of APs delivered at 20 Hz were used to evoke EPSPs in the second cell. The amplitude of the second EPSP was divided by the first and normalized over the baseline period (data from 5/5 pairs in which PPR was monitored).
B - Average traces of the second EPSP (30 sweeps) recorded during the baseline period and following potentiation (left) and depression (right). The amplitude of the first pulse is scaled (not shown) to highlight the change in PPR.
C - Plots of the change in the coefficient of variation (CV2) following TBS plotted against post-TBS normalized LE amplitude for potentiation (C1) and depressing cases (C2) (open triangle represents connection shown in 5.4B1, open diamond represents connection in 5.4B2; bars represent standard error).

Chapter 6 – General discussion

This thesis aimed to clarify the modes and mechanisms of communication available to mitral cells which are exclusive to the intraglomerular network. The glomerular networks are thought to play a modular role in olfactory bulb processing and thus understanding the way its components wire and communicate is crucial to a better understanding of the processing taking place within this structure. I have shown that several modes of communication are available to mitral cells and that these are relatively independent from each other. The fact that lateral excitation is unreliable unlike self excitation and electrical coupling suggests that it cannot be explained as a combination of the latter two as proposed by previous studies (Schoppa & Westbrook, 2002; Christie & Westbrook, 2006). This notion is more strongly substantiated by the fact that self and lateral excitation are mediated by distinct sub-classes of glutamate receptors and thus rely on different mechanisms and have separate roles in olfactory processing. In addition the short term dynamics of self excitation is different from that of lateral excitation. These results thus provide new insights on the functional connectivity of the olfactory bulb and mechanisms of intraglomerular mitral cell computation.

Self excitation of mitral cells

The role of self excitation in olfactory processing is at very least puzzling. Is it simply an epiphenomenon of the close proximity of glutamate releasing sites and glutamate receptors?

This work has provided insight onto the properties of self excitation but clearly much remains unknown about this type of communication. I have shown that self excitation is a ubiquitous trait of mitral cells; under normal physiological conditions it is expected that upon mitral cell activation, self excitation substantially contributes further to mitral cell depolarization, particularly within the apical tuft. This could

facilitate further action potential discharge in situations in which sensory drive might not be enough to elicit more action potentials (Nicoll & Jahr, 1982; Aroniadou-Anderjaska *et al.*, 1999; Isaacson, 1999; Friedman & Strowbridge, 2000; Salin *et al.*, 2001). Thus self excitation could be a mechanism to increase the gain of mitral cells in situations of low sensory neuron activity (e.g low concentration or low affinity for a particular odorant). One interesting possibility is that sensory drive alone could provide enough depolarization to activate enough voltage sensitive channels to allow the release of glutamate from the tuft (and thus self and/or lateral excitation) without the need of a backpropagating action potential. Calcium influx through calcium permeable AMPA receptors has been shown to mediate neurotransmitter release in the retina (Chavez *et al.*, 2006). This could dissociate signaling at the glomerular level from somatic output which could substantially decrease the time necessary for action potential discharge and recruitment of lateral (and recurrent) inhibition.

The fact that self excitation is mediated by calcium permeable receptors raises further questions and possibilities for its role in olfactory processing. Calcium is one of the most potent intracellular signals to several intracellular pathways and in addition is a key player in vesicular release. Although it is clear that lateral transmission and self excitation must be fundamentally different forms of chemical transmission it would be interesting to investigate if these two forms of signaling could cross-talk by the means of coupling through intracellular signaling pathways or calcium sensitive channels. For instance the calcium from self excitation could activate intracellular pathways that in certain conditions could directly affect the release properties for lateral excitation (Isaacson & Murphy, 2001) or mitral cell excitability in general. The fact that lateral excitation showed little sensitivity to CP-AMPA blockers suggests that self excitation does not have a direct influence on dendritic release. However my experiments were performed with the use of a single action potential and while in the presence of picrotoxin. It remains to be determined whether self excitation cross-talk with lateral excitation can occur during bursts of

mitral cell activity or when inhibition is intact. Perhaps during bursts of action potentials the rise in calcium concentration would more likely reach levels that evoke widespread events in the apical tuft. Another interesting, and thus far, unaddressed question is whether self excitation itself can express the same kind of plasticity observed for lateral excitation. My data supports a presynaptic involvement in the observed changes in efficacy (Faber & Korn, 1991), whether these changes are reflected at all release sites in the tuft or if it is something more synapse specific like observed in other regions of the brain remains unknown. In fact the anatomical compartmentalization of self and lateral excitation is a possibility that also remains unexplored. However the contrasting short term dynamic profiles of these two modes of communication suggests that indeed the release sites involved might be different (Zucker & Regehr, 2002). Given these mentioned reasons it would be highly speculative to expect that self excitation is regulated in the same fashion as lateral excitation but it is a possibility. This hypothesis would be experimentally hard to test given the long duration of the plasticity experiments and the added time necessary for the application of glutamate receptor antagonists required to study self excitation. Such increase in experiment duration when combined with the observed sensitivity of self excitation to V_{rest} fluctuations would drastically limit the number of data points available for the assessment of changes in efficacy of self excitation. One interesting possibility though would be that self excitation itself could be the feedback signal that triggers the plasticity induction. CP-AMPA receptors have been implicated in several NMDA-receptor independent mechanisms of synaptic plasticity (Mahanty & Sah, 1998; Cull-Candy *et al.*, 2006; Isaac *et al.*, 2007). Performing the TBS induction protocol in the presence of NAS or any other CP-AMPA specific blocker would thus allow the assessment of whether these receptors and in particular self excitation plays a role in the induction of lateral excitation plasticity.

The fact that self excitation is exclusively mediated by CP-AMPA also raises some questions regarding what might be the anatomical substrate of self excitation.

Assuming spill-over as the mechanism then either CP-AMPA receptors have dramatically high affinities for glutamate in order to explain their exclusive activation or these receptors are anatomically segregated from calcium impermeable receptors in a way that puts them in closer vicinity to the self-release sites. The first is unlikely given that affinity for neurotransmitter is not thought to be drastically influenced by the GluR2 subunit composition in AMPA receptors although this subunit drastically changes other biophysical properties of these receptors (Isaac *et al.*, 2007; Kessler *et al.*, 2008). The latter would imply a very specific targeting of the distribution of glutamate receptors in mitral cells such that extra-synaptic CP-AMPA receptors would be positioned very close to self release sites and calcium impermeable AMPA receptors very close to neighbor mitral cells release sites. Such an arrangement would require a sophisticated receptor targeting mechanism which would imply a clear specification of structures for self and lateral excitation. The lack of correlation with gross parameters of mitral cell morphology and self excitation argue against an even distribution of release sites and receptors but rather that more specialized structures are involved. It is however possible that the glomerular milieu (Kosaka & Kosaka, 2005a) provides conditions that are favourable to spill over, but together with the observed generally large amplitude of self excitation it seems more likely, although speculative that this might not be the case.

Electrical coupling within the glomerular network of mitral cells

This work has extended on the notion that intraglomerular mitral cells are electrically coupled and provided evidence that all of those cells that project to the same glomerulus are interconnected forming a large ensemble of cells (Schoppa & Westbrook, 2001; Christie & Westbrook, 2006). Here I have shown that electrical coupling is unlikely to be the sole mediator of lateral excitation (Christie & Westbrook, 2006). However this does not mean that it can't contribute to lateral transmission in general but rather suggests that it contributes both to excitation and inhibition. Depending on what state is dominating the presynaptic cell when

compared to a postsynaptic one, excitation of one cell comes at the "cost" of the hyperpolarization of another. In addition both excitation and inhibition should be efficiently transmitted (apart from suprathreshold signals) and thus this mode of communication cannot commit to the reliable single action potential induced lateral excitation described here. However by effectively connecting the whole mitral cell network it should be a very efficient mechanism for synchronizing the whole glomerular network (Schoppa & Westbrook, 2001; Migliore *et al.*, 2005). Gap junctions are very effective in the generation and propagation of oscillations across networks of interconnected cells as it has been shown in several regions in the CNS (Connors & Long, 2004; Hormuzdi *et al.*, 2004). In particular in the olfactory bulb oscillations have been shown to occur and proposed to play a crucial role in olfactory processing (Laurent, 1996; MacLeod & Laurent, 1996; Wehr & Laurent, 1996; Margrie & Schaefer, 2003). Thus the ubiquitous expression of gap junctions within the intraglomerular network might be one mechanism among others to increase synchrony among the principal cells that belong to the same module.

Lateral excitation of intraglomerular mitral cells

Several lines of evidence support the existence of a direct chemical connection between mitral cells that share the same intraglomerular network. As discussed previously other studies had suggested that an excitatory connection between mitral cells would rely exclusively on the coupling of self excitatory potentials via gap junctions (Schoppa & Westbrook, 2002; Christie *et al.*, 2005; Christie & Westbrook, 2006). While it must be inevitable that gap junctions (combined with self excitation) contribute to lateral transmission, as mentioned above, given their very own nature gap junctions cannot provide an exclusively excitatory connection. As such gap junctions can contribute to lateral excitation as much as they can contribute to inhibition between mitral cells, and thus lateral balancing would probably be a more appropriate description for their role (Hormuzdi *et al.*, 2004). Under physiological temperature and synaptic transmission/reception conditions, the amount of self

excitation arising from a single action potential combined with the extent of electrical coupling between mitral cells is not sufficient to account for the observed amount of lateral excitation. In particular it is unlikely to contribute reliably to excitation in a scenario in which the presynaptic cell's AHP and inhibitory currents are presumably coupled with a similar efficiency.

Previous studies had reported a correlation between the integral of lateral excitation evoked by bursts of action potentials at very high frequencies (125 - 360 Hz) and the observed coupling coefficient (Christie & Westbrook, 2006). In those studies it remained unclear if this correlation was verified for both or only one of the two possible connections. In this study single action potentials were used and lateral excitation amplitudes were found to not correlate with electrical coupling. My analysis broke down each individual connection for each pair of mitral cells to demonstrate that the peak amplitude of lateral excitation does not correlate with the coupling coefficients within the same connections. In addition for a subset of these connections I was able to directly compare the relationship between the amplitudes of electrical coupling, self excitation and lateral excitation and observed no relationship between them.

Some additional experimental differences between the studies could account for part or whole of the differences in the obtained results. The animals used by Christie & Westbrook (2006) were younger and it is possible that gap junctions might be developmentally regulated and that their contribution to lateral excitation is increased in earlier stages of development. In such a scenario chemical lateral excitation would either be diminished or even absent and thus lateral transmission would be solely due to electrical coupling of self excitatory potentials. Another difference is that a higher concentration of extracellular calcium was used previously (Christie & Westbrook,2006; 2.5 mM vs 2 mM in my experiments). This could influence self-excitation two-fold; by increasing release probability which could result in enhanced self excitation and by increasing the driving force for

calcium currents via CP-AMPA receptors, leading to an increased contribution of self excitation over chemical lateral excitation to the overall lateral transmission between mitral cells. Another striking inconsistency comes from the fact that lateral excitation was absent in the Cx36 KO mouse. One possible explanation is that gap junctions are required for the specification of the synapses responsible for lateral excitation. Adaptation of the connectivity has been shown in inferior olive in Cx36 deficient mice (De Zeeuw *et al.*, 2003) similarly it is possible that during development the synaptic organization of the olfactory bulb adapts in order to compensate for the lack of electrical coupling.

The most direct evidence for the functional distinction between lateral and self excitation comes form the observation that they are in fact mediated by different subtypes of AMPA receptors and thus could be pharmacologically dissected from each other. Given this observation lateral excitation must be an independent "channel" for mitral cell– mitral cell communication which serves its own role in olfactory processing. Lateral excitation has been proposed to be a means of increasing synchrony between the populations of mitral cells (Schoppa & Westbrook, 2001; Migliore *et al.*, 2005) and/or generating large spontaneous events (LLDs) which are also remarkably synchronous in mitral cells (Carlson *et al.*, 2000). The notion that self excitation and lateral excitation are distinct mechanisms is further extended by the observation that their short term dynamic profiles are different (Zucker, 1973; Zucker & Regehr, 2002).

One other possible benefit of lateral excitation is that it could drive mitral cells in the same glomerular network closer to action potential threshold. In situations in which odour concentration is low excitation could build up on incoming sensory drive and make mitral cells, that otherwise would not discharge an action potential, to fire one. Unitary lateral excitation amplitudes reported here are small, and although mitral cell resting membrane potential is typically depolarized and close to threshold, lateral excitation from a single mitral cell would be unlikely to be sufficient to evoke

an action potential. However considering that mitral cell might receive inputs from several of its intraglomerular partners; such inputs should add to provide considerable depolarization that would significantly contribute to mitral cell output. In situations in which sensory drive is enough to reliably and strongly activate mitral cells, recurrent inhibition could efficiently kick in and dampen the activation (by counteracting lateral excitation) keeping mitral cells close to the relatively fixed firing rate that has been observed in vivo (Cang & Isaacson, 2003; Margrie & Schaefer, 2003). This would enhance the dynamic range of odour concentrations at which mitral cells are able to code for odors.

The data obtained here however provide a clue as to the function of lateral excitation. Lateral excitation provides the olfactory bulb with a means to modulate the communication between intraglomerular mitral cells. The exquisite sensitivity of lateral excitation to TBS suggests that changes in efficacy might serve a homeostatic role in the flow of sensory drive via the glomerular module by adjusting the gain of individual mitral cells. Based on the data presented here the likelihood of a mitral cell being linked by chemical transmission to any of its intraglomerular partners is about 62%. Taking into account that plasticity was successfully induced with action potentials being evoked on the presynaptic cell and there is a presynaptic expression site, it is likely that theta patterned mitral cell activation modulates transmission with several of its intraglomerular partners. The outcome of this modulation should depend on the individual connections. At several synapses where plasticity expresses presynaptically, it has often been proposed that a retrograde signal could provide the link between the two cells. Several candidate molecules have been proposed to mediate such signaling including, nitric oxide, arachidonic acid and endocannabinoids (Malenka & Bear, 2004). Whether such retrograde signaling occurs between intraglomerular mitral cells remains unexplored.

It would also be interesting to investigate how postsynaptic spiking might impact on the expression of plasticity. *In-vivo* and during sniffing it has been proposed that the intraglomerular ensemble of mitral cells transmits highly synchronous information to downstream targets (Suzuki & Bekkers, 2006). Thus it would be interesting to find out whether in conditions in which both mitral cells fire action potentials the TBS induced changes in efficacy would be bypassed, unchanged or enhanced. As mentioned before the interval between pre and post-synaptic spiking has been shown to have a profound influence on the changes in synaptic efficacy (Markram *et al.*, 1997; Sjostrom *et al.*, 2001; Sjostrom & Hausser, 2006). It remains to be shown if such a mechanism could occur between mitral cells or in the olfactory bulb in general. Studies have indicated that LTP induced at CA1 synapses can be reversed by further activation of the synapse (Abraham & Huggett, 1997; Huang *et al.*, 2001) My experiments suggest that such changes last for tens of minutes but could further activity reset the previously induced change? Can a connection be either depressed or potentiated enough that a following round of TBS would invert the direction of the change? This would be expected if TBS is to have a normalizing action on lateral excitation. Answer to these questions will provide more insight into the role of lateral excitation in the context of the whole glomerular network and olfactory bulb processing.

However the finding that lateral excitation can express changes in efficacy when stimulated with *in-vivo* relevant patterns of activity provides for the first time evidence that, similar to what has been observed for axo-dendritic synapses, dendrodendritic transmission can express long term increases and decreases in its efficacy. Dendrites were traditionally seen merely as receivers of information but the increasing knowledge on their function and properties has changed that notion in the past years. Nowadays these structures are seen as complex and active players in neural processing and in particular in the olfactory bulb (though not exclusively) as transmitters of information (Margrie & Urban, 2007). Here I show that release from these structures can be modulated much like in their axonic counterparts. This

raises the possibility that other dendrodentric synapses (i.e. mitral cell to granule cell synapse) might also express such forms of plasticity which could act as a substrate for phenomena such as olfactory memory (Kaba *et al.*, 1994; Sullivan & Wilson, 2003), sensory adaptation (Li, 1990), tuning of receptive fields or discrimination learning (Wilson *et al.*, 2004).

The anatomical correlates of lateral excitation remain to be identified. It is clear the locus is in the apical tuft. I have never observed lateral excitation in cells in which one or both apical tufts had been amputated by the slicing procedure. Furthermore the minimum dendritic distance suggests that lateral dendrites are in general too distant (and much greater than the tuft processes) to mediate any form of communication between mitral cells under my experimental conditions. Previous work from Urban and Sakmann (2002) in which dendritic recordings from intraglomerular pairs were performed has also indicated that the source of AMPA-mediated lateral excitation is in the apical tuft.

However the somewhat crude analysis of morphology performed here does not provide the necessary resolution to identify putative contacts within the tuft. The morphological analysis performed here does suggest that the likelihood and strength of lateral excitation are reasonably detached from macroscopic features of tuft morphometry. This does not mean to imply that lateral excitation is absolutely independent of morphological features since it obviously should result from dendritic opposition. The tools available for this study lacked resolution for the search of these sites. Unlike in other studies I have not distinctively labeled the two mitral cells (pre/post) which compromises the ability to discriminate unambiguously between the two cells in regions were they are in close contact (Christie & Westbrook, 2006). This limitation could be addressed using different dyes though I have concerns regarding the resolution of neurolucida or light microscopy in general to report genuine contacts and in particular to distinguish between a gap junction contact from a chemical transmission contact. Nevertheless it will be important to

examine the ultra structure of recorded mitral cells to investigate this issue. In order to identify the precise anatomical substrates for LE, quantification beyond light microscopy resolution will be necessary. Given the number of mitral cells per glomerulus and their elaborate tuft morphologies, ultrastructural analysis of the entire glomerulus would ideally be required (Denk & Horstmann, 2004). The observation that LE is heterogeneously expressed and independent of the overall size of mitral cell tufts suggests that the specification of these connections may be dependent on developmental features and/or history of activity, the rules for which remain undetermined. Such an impact of activity during developmental stages in the arrangement of neural networks has been well studied in the visual cortex in which sensory activity shapes ocular dominance columns (reviewed in Katz & Crowley, 2002).

Homeostatic mechanisms within the glomerular network

A growing body of evidence suggests that the nervous systems is equipped with a number of mechanisms to ensure that stability of neural networks is not compromised by the fact that synaptic strength can be modulated. For instance an increase in the synaptic strength at synapses to a given neuron would result in increasing the overall excitatory drive to this cell that would likely cause it to adopt different firing rates and ultimately change the dynamics of the network. Compensatory mechanisms range from scaling of synaptic input (Turrigiano *et al.*, 1994) to changes in intrinsic excitability (Xu *et al.*, 2005). Such adaptations are thought to allow individual cells to cope with changes in synaptic input and are thus referred to as homeostatic mechanisms (reviewed in Turrigiano, 2007; Nelson & Turrigiano, 2008; Turrigiano, 2008). The data presented here suggests that the intraglomerular circuitry is equipped with several self-normalizing mechanisms that could parallel such mechanisms. On a shorter time scale the short-term dynamics of self excitation, as discussed above/chapter 3 seems to be accommodating in order to be less efficient at higher frequencies and stronger at lower frequencies. This

could be part of a mechanism of normalizing the mitral cell firing rate which has been reported to be reasonably fixed at within 50-60 Hz.

The observed variability of lateral excitation and in particular its remarkable sensitivity to TBS suggests that this connection could also serve a homeostatic role. TBS effectively normalized the lateral excitation efficacy in a manner that was strictly dependent on the initial state of the synapse. Stronger synapses become depressed and weaker ones potentiated in a way that suggests that these are normalized in order to keep fixed the overall excitation that flows via the glomerular network. This could act as a compensatory mechanism to overcome situations in which a fraction of the mitral cells within a glomerulus receive deficient input from sensory neurons. The deficient input may occur due to the high sensory neuron regeneration and re-innervation rate (Barber, 1982; Kern *et al.*, 2004). Lateral excitation could provide a boost to the network that would facilitate action potential discharge in such neurons in order to ensure synchrony within the mitral cell ensemble. Such mechanisms can increase the fidelity of sensory transmission and processing in the olfactory system. For instance in the fly antennal lobe (equivalent to the vertebrate olfactory bulb) it has recently been shown that a homeostatic mechanism matches the strength of sensory input to the size and input resistance of the postsynaptic cell (Kazama & Wilson, 2008).

Bibliography:

Abraham NM, Spors H, Carleton A, Margrie TW, Kuner T & Schaefer AT. (2004). Maintaining accuracy at the expense of speed: stimulus similarity defines odor discrimination time in mice. *Neuron* **44,** 865-876.

Abraham WC & Huggett A. (1997). Induction and reversal of long-term potentiation by repeated high-frequency stimulation in rat hippocampal slices. *Hippocampus* **7,** 137-145.

Arevian AC, Kapoor V & Urban NN. (2008). Activity-dependent gating of lateral inhibition in the mouse olfactory bulb. *Nat Neurosci* **11,** 80-87.

Aroniadou-Anderjaska V, Ennis M & Shipley MT. (1999). Dendrodendritic recurrent excitation in mitral cells of the rat olfactory bulb. *J Neurophysiol* **82,** 489-494.

Aungst JL, Heyward PM, Puche AC, Karnup SV, Hayar A, Szabo G & Shipley MT. (2003). Centre-surround inhibition among olfactory bulb glomeruli. *Nature* **426,** 623-629.

Bailey CH & Kandel ER. (2008). Synaptic remodeling, synaptic growth and the storage of long-term memory in Aplysia. *Progress in brain research* **169,** 179-198.

Balu R, Larimer P & Strowbridge BW. (2004). Phasic stimuli evoke precisely timed spikes in intermittently discharging mitral cells. *J Neurophysiol* **92,** 743-753.

Barber PC. (1982). Neurogenesis and regeneration in the primary olfactory pathway of mammals. *Bibliotheca anatomica,* 12-25.

Barria A, Muller D, Derkach V, Griffith LC & Soderling TR. (1997). Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* **276,** 2042-2045.

Bekkers JM & Hausser M. (2007). Targeted dendrotomy reveals active and passive contributions of the dendritic tree to synaptic integration and neuronal output. *Proceedings of the National Academy of Sciences of the United States of America* **104,** 11447-11452.

Belluscio L, Lodovichi C, Feinstein P, Mombaerts P & Katz LC. (2002). Odorant receptors instruct functional circuitry in the mouse olfactory bulb. *Nature* **419,** 296-300.

Bennett MV. (2000a). Electrical synapses, a personal perspective (or history). *Brain research* **32,** 16-28.

Bennett MV. (2000b). Seeing is relieving: electrical synapses between visualized neurons. *Nat Neurosci* **3,** 7-9.

Bennett MV & Zukin RS. (2004). Electrical coupling and neuronal synchronization in the Mammalian brain. *Neuron* **41,** 495-511.

Beyer EC, Paul DL & Goodenough DA. (1990). Connexin family of gap junction proteins. *The Journal of membrane biology* **116,** 187-194.

Bhandawat V, Reisert J & Yau KW. (2005). Elementary response of olfactory receptor neurons to odorants. *Science* **308,** 1931-1934.

Bischofberger J & Jonas P. (1997). Action potential propagation into the presynaptic dendrites of rat mitral cells. *The Journal of physiology* **504 (Pt 2),** 359-365.

Bjorklund A & Lindvall O. (1975). Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. *Brain Res* **83,** 531-537.

Blakemore LJ, Resasco M, Mercado MA & Trombley PQ. (2006). Evidence for Ca(2+)-permeable AMPA receptors in the olfactory bulb. *Am J Physiol Cell Physiol* **290,** C925-935.

Bliss TV & Lomo T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* **232,** 331-356.

Borst JG & Sakmann B. (1996). Calcium influx and transmitter release in a fast CNS synapse. *Nature* **383,** 431-434.

Bozza T, Feinstein P, Zheng C & Mombaerts P. (2002). Odorant receptor expression defines functional units in the mouse olfactory system. *J Neurosci* **22,** 3033-3043.

Bozza T, McGann JP, Mombaerts P & Wachowiak M. (2004). In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* **42,** 9-21.

Bozza TC & Mombaerts P. (2001). Olfactory coding: revealing intrinsic representations of odors. *Curr Biol* **11,** R687-690.

Brody CD & Hopfield JJ. (2003). Simple networks for spike-timing-based computation, with application to olfactory processing. *Neuron* **37,** 843-852.

Broome BM, Jayaraman V & Laurent G. (2006). Encoding and decoding of overlapping odor sequences. *Neuron* **51,** 467-482.

Bruzzone R, Hormuzdi SG, Barbe MT, Herb A & Monyer H. (2003). Pannexins, a family of gap junction proteins expressed in brain. *Proceedings of the National Academy of Sciences of the United States of America* **100,** 13644-13649.

Buck L & Axel R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65,** 175-187.

Buhl DL, Harris KD, Hormuzdi SG, Monyer H & Buzsaki G. (2003). Selective impairment of hippocampal gamma oscillations in connexin-36 knock-out mouse in vivo. *J Neurosci* **23,** 1013-1018.

Cang J & Isaacson JS. (2003). In vivo whole-cell recording of odor-evoked synaptic transmission in the rat olfactory bulb. *J Neurosci* **23,** 4108-4116.

Carlson GC, Shipley MT & Keller A. (2000). Long-lasting depolarizations in mitral cells of the rat olfactory bulb. *J Neurosci* **20,** 2011-2021.

Charpak S, Mertz J, Beaurepaire E, Moreaux L & Delaney K. (2001). Odor-evoked calcium signals in dendrites of rat mitral cells. *Proceedings of the National Academy of Sciences of the United States of America* **98,** 1230-1234.

Chavez AE, Singer JH & Diamond JS. (2006). Fast neurotransmitter release triggered by Ca influx through AMPA-type glutamate receptors. *Nature* **443,** 705-708.

Chen WR, Midtgaard J & Shepherd GM. (1997). Forward and backward propagation of dendritic impulses and their synaptic control in mitral cells. *Science* **278,** 463-467.

Christie JM, Bark C, Hormuzdi SG, Helbig I, Monyer H & Westbrook GL. (2005). Connexin36 mediates spike synchrony in olfactory bulb glomeruli. *Neuron* **46,** 761-772.

Christie JM & Westbrook GL. (2003). Regulation of backpropagating action potentials in mitral cell lateral dendrites by A-type potassium currents. *J Neurophysiol* **89,** 2466-2472.

Christie JM & Westbrook GL. (2006). Lateral excitation within the olfactory bulb. *J Neurosci* **26,** 2269-2277.

Citri A & Malenka RC. (2008). Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology* **33,** 18-41.

Cleland TA & Linster C. (2002). How synchronization properties among second-order sensory neurons can mediate stimulus salience. *Behav Neurosci* **116,** 212-221.

Connors BW & Long MA. (2004). Electrical synapses in the mammalian brain. *Annu Rev Neurosci* **27,** 393-418.

Cull-Candy S, Kelly L & Farrant M. (2006). Regulation of Ca2+-permeable AMPA receptors: synaptic plasticity and beyond. *Current opinion in neurobiology* **16,** 288-297.

Davison IG, Boyd JD & Delaney KR. (2004). Dopamine inhibits mitral/tufted--> granule cell synapses in the frog olfactory bulb. *J Neurosci* **24,** 8057-8067.

De Zeeuw CI, Chorev E, Devor A, Manor Y, Van Der Giessen RS, De Jeu MT, Hoogenraad CC, Bijman J, Ruigrok TJ, French P, Jaarsma D, Kistler WM, Meier C, Petrasch-Parwez E, Dermietzel R, Sohl G, Gueldenagel M, Willecke K & Yarom Y. (2003). Deformation of network connectivity in the inferior olive of connexin 36-deficient mice is compensated by morphological and electrophysiological changes at the single neuron level. *J Neurosci* **23,** 4700-4711.

Del Castillo J & Katz B. (1954a). Electrophoretic application of acetylcholine to the two sides of the end-plate membrane. *The Journal of physiology* **125,** 16-17P.

Del Castillo J & Katz B. (1954b). Quantal components of the end-plate potential. *J Physiol* **124,** 560-573.

Denk W & Horstmann H. (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biol* **2,** e329.

Djurisic M, Antic S, Chen WR & Zecevic D. (2004). Voltage imaging from dendrites of mitral cells: EPSP attenuation and spike trigger zones. *J Neurosci* **24,** 6703-6714.

Djurisic M, Popovic M, Carnevale N & Zecevic D. (2008). Functional structure of the mitral cell dendritic tuft in the rat olfactory bulb. *J Neurosci* **28,** 4057-4068.

Drake CT, Terman GW, Simmons ML, Milner TA, Kunkel DD, Schwartzkroin PA & Chavkin C. (1994). Dynorphin opioids present in dentate granule cells may function as retrograde inhibitory neurotransmitters. *J Neurosci* **14,** 3736-3750.

Duchamp-Viret P, Duchamp A & Chaput MA. (2000). Peripheral odor coding in the rat and frog: quality and intensity specification. *J Neurosci* **20,** 2383-2390.

Dudek SM & Bear MF. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences of the United States of America* **89,** 4363-4367.

Eccles JC. (1982). The synapse: from electrical to chemical transmission. *Annu Rev Neurosci* **5,** 325-339.

Edwards FA. (1995). Anatomy and electrophysiology of fast central synapses lead to a structural model for long-term potentiation. *Physiol Rev* **75,** 759-787.

Egger V & Urban NN. (2006). Dynamic connectivity in the mitral cell-granule cell microcircuit. *Semin Cell Dev Biol* **17,** 424-432.

Faber DS & Korn H. (1991). Applicability of the coefficient of variation method for analyzing synaptic plasticity. *Biophys J* **60,** 1288-1294.

Fatt P & Katz B. (1952). Spontaneous subthreshold activity at motor nerve endings. *The Journal of physiology* **117,** 109-128.

Foster KA, Kreitzer AC & Regehr WG. (2002). Interaction of postsynaptic receptor saturation with presynaptic mechanisms produces a reliable synapse. *Neuron* **36,** 1115-1126.

Freund TF, Katona I & Piomelli D. (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* **83,** 1017-1066.

Friedman D & Strowbridge BW. (2000). Functional role of NMDA autoreceptors in olfactory mitral cells. *J Neurophysiol* **84,** 39-50.

Friedman D & Strowbridge BW. (2003). Both electrical and chemical synapses mediate fast network oscillations in the olfactory bulb. *J Neurophysiol* **89,** 2601-2610.

Fuss SH & Korsching SI. (2001). Odorant feature detection: activity mapping of structure response relationships in the zebrafish olfactory bulb. *J Neurosci* **21,** 8396-8407.

Galan RF, Fourcaud-Trocme N, Ermentrout GB & Urban NN. (2006). Correlation-induced synchronization of oscillations in olfactory bulb neurons. *J Neurosci* **26,** 3646-3655.

Galarreta M, Erdelyi F, Szabo G & Hestrin S. (2004). Electrical coupling among irregular-spiking GABAergic interneurons expressing cannabinoid receptors. *J Neurosci* **24,** 9770-9778.

Galarreta M & Hestrin S. (1999). A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature* **402,** 72-75.

Gibson JR, Beierlein M & Connors BW. (1999). Two networks of electrically coupled inhibitory neurons in neocortex. *Nature* **402,** 75-79.

Glitsch M, Llano I & Marty A. (1996). Glutamate as a candidate retrograde messenger at interneurone-Purkinje cell synapses of rat cerebellum. *J Physiol* **497 (Pt 2),** 531-537.

Halabisky B & Strowbridge BW. (2003). Gamma-frequency excitatory input to granule cells facilitates dendrodendritic inhibition in the rat olfactory Bulb. *J Neurophysiol* **90,** 644-654.

Hardingham NR, Hardingham GE, Fox KD & Jack JJ. (2007). Presynaptic efficacy directs normalization of synaptic strength in layer 2/3 rat neocortex after paired activity. *J Neurophysiol* **97,** 2965-2975.

Harsanyi K & Mangel SC. (1992). Activation of a D2 receptor increases electrical coupling between retinal horizontal cells by inhibiting dopamine release. *Proc Natl Acad Sci U S A* **89,** 9220-9224.

Hausser M, Stuart G, Racca C & Sakmann B. (1995). Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons. *Neuron* **15,** 637-647.

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

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC & Malinow R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287,** 2262-2267.

Herve JC & Sarrouilhe D. (2006). Protein phosphatase modulation of the intercellular junctional communication: importance in cardiac myocytes. *Prog Biophys Mol Biol* **90,** 225-248.

Hestrin S & Galarreta M. (2005). Electrical synapses define networks of neocortical GABAergic neurons. *Trends Neurosci* **28,** 304-309.

Holtmaat A, Wilbrecht L, Knott GW, Welker E & Svoboda K. (2006). Experience-dependent and cell-type-specific spine growth in the neocortex. *Nature* **441,** 979-983.

Horikawa K & Armstrong WE. (1988). A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. *J Neurosci Methods* **25,** 1-11.

Hormuzdi SG, Filippov MA, Mitropoulou G, Monyer H & Bruzzone R. (2004). Electrical synapses: a dynamic signaling system that shapes the activity of neuronal networks. *Biochim Biophys Acta* **1662,** 113-137.

Hormuzdi SG, Pais I, LeBeau FE, Towers SK, Rozov A, Buhl EH, Whittington MA & Monyer H. (2001). Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice. *Neuron* **31,** 487-495.

Huang CC, Liang YC & Hsu KS. (2001). Characterization of the mechanism underlying the reversal of long term potentiation by low frequency stimulation at hippocampal CA1 synapses. *J Biol Chem* **276,** 48108-48117.

Isaac JT, Ashby M & McBain CJ. (2007). The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron* **54,** 859-871.

Isaacson JS. (1999). Glutamate spillover mediates excitatory transmission in the rat olfactory bulb. *Neuron* **23,** 377-384.

Isaacson JS & Murphy GJ. (2001). Glutamate-mediated extrasynaptic inhibition: direct coupling of NMDA receptors to Ca(2+)-activated K+ channels. *Neuron* **31,** 1027-1034.

Isaacson JS & Strowbridge BW. (1998). Olfactory reciprocal synapses: dendritic signaling in the CNS. *Neuron* **20,** 749-761.

Isaacson JS & Vitten H. (2003). GABA(B) receptors inhibit dendrodendritic transmission in the rat olfactory bulb. *J Neurosci* **23,** 2032-2039.

Isaacson JS & Walmsley B. (1995). Counting quanta: direct measurements of transmitter release at a central synapse. *Neuron* **15,** 875-884.

Jahr CE & Nicoll RA. (1980). Dendrodendritic inhibition: demonstration with intracellular recording. *Science* **207,** 1473-1475.

Johnson BA & Leon M. (2007). Chemotopic odorant coding in a mammalian olfactory system. *J Comp Neurol* **503,** 1-34.

Jones MV & Westbrook GL. (1996). The impact of receptor desensitization on fast synaptic transmission. *Trends in neurosciences* **19,** 96-101.

Kaba H, Hayashi Y, Higuchi T & Nakanishi S. (1994). Induction of an olfactory memory by the activation of a metabotropic glutamate receptor. *Science* **265,** 262-264.

Kaba H & Keverne EB. (1988). The effect of microinfusions of drugs into the accessory olfactory bulb on the olfactory block to pregnancy. *Neuroscience* **25,** 1007-1011.

Kamiya H & Zucker RS. (1994). Residual Ca2+ and short-term synaptic plasticity. *Nature* **371,** 603-606.

Kandel ER. (2001). The molecular biology of memory storage: a dialogue between genes and synapses. *Science* **294,** 1030-1038.

Katz LC & Crowley JC. (2002). Development of cortical circuits: lessons from ocular dominance columns. *Nature reviews* **3,** 34-42.

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

Kazama H & Wilson RI. (2008). Homeostatic matching and nonlinear amplification at identified central synapses. *Neuron* **58,** 401-413.

Kepecs A, Uchida N & Mainen ZF. (2006). The sniff as a unit of olfactory processing. *Chem Senses* **31,** 167-179.

Kern RC, Conley DB, Haines GK, 3rd & Robinson AM. (2004). Pathology of the olfactory mucosa: implications for the treatment of olfactory dysfunction. *The Laryngoscope* **114,** 279-285.

Kessler M, Suzuki E, Montgomery K & Arai AC. (2008). Physiological significance of high- and low-affinity agonist binding to neuronal and recombinant AMPA receptors. *Neurochemistry international* **52,** 1383-1393.

Korn H & Faber DS. (1991). Quantal analysis and synaptic efficacy in the CNS. *Trends Neurosci* **14,** 439-445.

Kosaka K & Kosaka T. (2005a). synaptic organization of the glomerulus in the main olfactory bulb: compartments of the glomerulus and heterogeneity of the periglomerular cells. *Anat Sci Int* **80,** 80-90.

Kosaka T & Kosaka K. (2003). Neuronal gap junctions in the rat main olfactory bulb, with special reference to intraglomerular gap junctions. *Neurosci Res* **45,** 189-209.

Kosaka T & Kosaka K. (2004). Neuronal gap junctions between intraglomerular mitral/tufted cell dendrites in the mouse main olfactory bulb. *Neurosci Res* **49,** 373-378.

Kosaka T & Kosaka K. (2005b). Intraglomerular dendritic link connected by gap junctions and chemical synapses in the mouse main olfactory bulb: electron microscopic serial section analyses. *Neuroscience* **131,** 611-625.

Koulakov A, Gelperin A & Rinberg D. (2007). Olfactory coding with all-or-nothing glomeruli. *J Neurophysiol* **98,** 3134-3142.

Landisman CE & Connors BW. (2005). Long-term modulation of electrical synapses in the mammalian thalamus. *Science* **310,** 1809-1813.

Larson J, Wong D & Lynch G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. *Brain Res* **368,** 347-350.

Laurent G. (1996). Dynamical representation of odors by oscillating and evolving neural assemblies. *Trends Neurosci* **19,** 489-496.

Li Z. (1990). A model of olfactory adaptation and sensitivity enhancement in the olfactory bulb. *Biol Cybern* **62,** 349-361.

Lledo PM, Alonso M & Grubb MS. (2006). Adult neurogenesis and functional plasticity in neuronal circuits. *Nat Rev Neurosci* **7,** 179-193.

Lomo T. (1968). Potentiation of monosynaptic EPSPs in cortical cells by single and repetitive afferent volleys. *J Physiol* **194,** 84-85P.

Long MA, Deans MR, Paul DL & Connors BW. (2002). Rhythmicity without synchrony in the electrically uncoupled inferior olive. *J Neurosci* **22,** 10898-10905.

Lowe G. (2002). Inhibition of backpropagating action potentials in mitral cell secondary dendrites. *J Neurophysiol* **88,** 64-85.

Ludwig M & Pittman QJ. (2003). Talking back: dendritic neurotransmitter release. *Trends Neurosci* **26,** 255-261.

Luo M & Katz LC. (2001). Response correlation maps of neurons in the mammalian olfactory bulb. *Neuron* **32,** 1165-1179.

Ma J & Lowe G. (2007). Calcium permeable AMPA receptors and autoreceptors in external tufted cells of rat olfactory bulb. *Neuroscience* **144,** 1094-1108.

Macdonald RL & Olsen RW. (1994). GABAA receptor channels. *Annu Rev Neurosci* **17,** 569-602.

MacLeod K & Laurent G. (1996). Distinct mechanisms for synchronization and temporal patterning of odor-encoding neural assemblies. *Science* **274,** 976-979.

Mahanty NK & Sah P. (1998). Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala. *Nature* **394,** 683-687.

Maher BJ & Westbrook GL. (2005). SK channel regulation of dendritic excitability and dendrodendritic inhibition in the olfactory bulb. *J Neurophysiol* **94,** 3743-3750.

Malenka RC. (1991). Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. *Neuron* **6,** 53-60.

Malenka RC & Bear MF. (2004). LTP and LTD: an embarrassment of riches. *Neuron* **44,** 5-21.

Malinow R, Mainen ZF & Hayashi Y. (2000). LTP mechanisms: from silence to four-lane traffic. *Curr Opin Neurobiol* **10,** 352-357.

Malinow R & Tsien RW. (1990). Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* **346,** 177-180.

Margrie TW, Sakmann B & Urban NN. (2001). Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb. *Proceedings of the National Academy of Sciences of the United States of America* **98,** 319-324.

Margrie TW & Schaefer AT. (2003). Theta oscillation coupled spike latencies yield computational vigour in a mammalian sensory system. *J Physiol* **546,** 363-374.

Margrie TW & Urban NN. (2007). Dendrites as transmiters. In *Dendrites*, ed. Stuart G, Hausser M & Spruston N, pp. 399-417.

Markram H, Lubke J, Frotscher M & Sakmann B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275,** 213-215.

Mayer ML. (2005). Glutamate receptor ion channels. *Current opinion in neurobiology* **15,** 282-288.

McMahon DG, Knapp AG & Dowling JE. (1989). Horizontal cell gap junctions: single-channel conductance and modulation by dopamine. *Proc Natl Acad Sci U S A* **86,** 7639-7643.

Meister M & Bonhoeffer T. (2001). Tuning and topography in an odor map on the rat olfactory bulb. *J Neurosci* **21,** 1351-1360.

Migliore M, Hines ML & Shepherd GM. (2005). The role of distal dendritic gap junctions in synchronization of mitral cell axonal output. *J Comput Neurosci* **18,** 151-161.

Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J & Axel R. (1996). Visualizing an olfactory sensory map. *Cell* **87,** 675-686.

Montague AA & Greer CA. (1999). Differential distribution of ionotropic glutamate receptor subunits in the rat olfactory bulb. *J Comp Neurol* **405,** 233-246.

Murphy GJ, Darcy DP & Isaacson JS. (2005). Intraglomerular inhibition: signaling mechanisms of an olfactory microcircuit. *Nat Neurosci* **8,** 354-364.

Nagerl UV, Eberhorn N, Cambridge SB & Bonhoeffer T. (2004). Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* **44,** 759-767.

Nelson SB & Turrigiano GG. (2008). Strength through diversity. *Neuron* **60,** 477-482.

Nicoll RA & Jahr CE. (1982). Self-excitation of olfactory bulb neurones. *Nature* **296,** 441-444.

O'Donnell P & Grace AA. (1993). Dopaminergic modulation of dye coupling between neurons in the core and shell regions of the nucleus accumbens. *J Neurosci* **13,** 3456-3471.

Ozawa S, Kamiya H & Tsuzuki K. (1998). Glutamate receptors in the mammalian central nervous system. *Progress in neurobiology* **54,** 581-618.

Parrish-Aungst S, Shipley MT, Erdelyi F, Szabo G & Puche AC. (2007). Quantitative analysis of neuronal diversity in the mouse olfactory bulb. *J Comp Neurol* **501,** 825-836.

Paulsen O & Sejnowski TJ. (2000). Natural patterns of activity and long-term synaptic plasticity. *Curr Opin Neurobiol* **10,** 172-179.

Pimentel DO & Margrie TW. (2008). Glutamatergic transmission and plasticity between olfactory bulb mitral cells. *The Journal of physiology.*

Pinching AJ & Powell TP. (1971a). The neuron types of the glomerular layer of the olfactory bulb. *J Cell Sci* **9,** 305-345.

Pinching AJ & Powell TP. (1971b). The neuropil of the glomeruli of the olfactory bulb. *J Cell Sci* **9,** 347-377.

Pinching AJ & Powell TP. (1971c). The neuropil of the periglomerular region of the olfactory bulb. *J Cell Sci* **9,** 379-409.

Pinching AJ & Powell TP. (1972). Experimental studies on the axons intrinsic to the glomerular layer of the olfactory bulb. *J Cell Sci* **10,** 637-655.

Price JL & Powell TP. (1970). The mitral and short axon cells of the olfactory bulb. *J Cell Sci* **7,** 631-651.

Rall W, Shepherd GM, Reese TS & Brightman MW. (1966). Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Exp Neurol* **14,** 44-56.

Redman S. (1990). Quantal analysis of synaptic potentials in neurons of the central nervous system. *Physiol Rev* **70,** 165-198.

Reyes A, Lujan R, Rozov A, Burnashev N, Somogyi P & Sakmann B. (1998). Target-cell-specific facilitation and depression in neocortical circuits. *Nat Neurosci* **1,** 279-285.

Reyher CK, Lubke J, Larsen WJ, Hendrix GM, Shipley MT & Baumgarten HG. (1991). Olfactory bulb granule cell aggregates: morphological evidence for interperikaryal electrotonic coupling via gap junctions. *J Neurosci* **11,** 1485-1495.

Rinberg D, Koulakov A & Gelperin A. (2006). Speed-accuracy tradeoff in olfaction. *Neuron* **51,** 351-358.

Rosser AE & Keverne EB. (1985). The importance of central noradrenergic neurones in the formation of an olfactory memory in the prevention of pregnancy block. *Neuroscience* **15,** 1141-1147.

Rozov A, Burnashev N, Sakmann B & Neher E. (2001). Transmitter release modulation by intracellular Ca2+ buffers in facilitating and depressing nerve terminals of pyramidal cells in layer 2/3 of the rat neocortex indicates a target cell-specific difference in presynaptic calcium dynamics. *The Journal of physiology* **531,** 807-826.

Sabatini BL & Regehr WG. (1999). Timing of synaptic transmission. *Annu Rev Physiol* **61,** 521-542.

Salin PA, Lledo PM, Vincent JD & Charpak S. (2001). Dendritic glutamate autoreceptors modulate signal processing in rat mitral cells. *J Neurophysiol* **85,** 1275-1282.

Sassoe-Pognetto M & Ottersen OP. (2000). Organization of ionotropic glutamate receptors at dendrodendritic synapses in the rat olfactory bulb. *J Neurosci* **20,** 2192-2201.

Sassoe-Pognetto M, Utvik JK, Camoletto P, Watanabe M, Stephenson FA, Bredt DS & Ottersen OP. (2003). Organization of postsynaptic density proteins and glutamate receptors in axodendritic and dendrodendritic synapses of the rat olfactory bulb. *J Comp Neurol* **463,** 237-248.

Schaefer AT, Angelo K, Spors H & Margrie TW. (2006). Neuronal oscillations enhance stimulus discrimination by ensuring action potential precision. *PLoS Biol* **4,** e163.

Schaefer AT & Margrie TW. (2007). Spatiotemporal representations in the olfactory system. *Trends Neurosci* **30,** 92-100.

Schoppa NE & Urban NN. (2003). Dendritic processing within olfactory bulb circuits. *Trends in neurosciences* **26,** 501-506.

Schoppa NE & Westbrook GL. (2001). Glomerulus-specific synchronization of mitral cells in the olfactory bulb. *Neuron* **31,** 639-651.

Schoppa NE & Westbrook GL. (2002). AMPA autoreceptors drive correlated spiking in olfactory bulb glomeruli. *Nat Neurosci* **5,** 1194-1202.

Scott JW, Acevedo HP & Sherrill L. (2006). Effects of concentration and sniff flow rate on the rat electroolfactogram. *Chem Senses* **31,** 581-593.

Shimshek DR, Bus T, Kim J, Mihaljevic A, Mack V, Seeburg PH, Sprengel R & Schaefer AT. (2005). Enhanced odor discrimination and impaired olfactory memory by spatially controlled switch of AMPA receptors. *PLoS Biol* **3,** e354.

Silver RA, Momiyama A & Cull-Candy SG. (1998). Locus of frequency-dependent depression identified with multiple-probability fluctuation analysis at rat climbing fibre-Purkinje cell synapses. *J Physiol* **510 (Pt 3),** 881-902.

Sjostrom PJ & Hausser M. (2006). A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. *Neuron* **51,** 227-238.

Sjostrom PJ, Turrigiano GG & Nelson SB. (2001). Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron* **32,** 1149-1164.

Sohl G, Maxeiner S & Willecke K. (2005). Expression and functions of neuronal gap junctions. *Nature reviews* **6,** 191-200.

Spors H & Grinvald A. (2002). Spatio-temporal dynamics of odor representations in the mammalian olfactory bulb. *Neuron* **34,** 301-315.

Stuart GJ, Dodt HU & Sakmann B. (1993). Patch-clamp recordings from the soma and dendrites of neurons in brain slices using infrared video microscopy. *Pflugers Arch* **423,** 511-518.

Sullivan RM, Stackenwalt G, Nasr F, Lemon C & Wilson DA. (2000). Association of an odor with activation of olfactory bulb noradrenergic beta-receptors or locus coeruleus stimulation is sufficient to produce learned approach responses to that odor in neonatal rats. *Behav Neurosci* **114,** 957-962.

Sullivan RM & Wilson DA. (2003). Molecular biology of early olfactory memory. *Learn Mem* **10,** 1-4.

Suzuki N & Bekkers JM. (2006). Neural coding by two classes of principal cells in the mouse piriform cortex. *J Neurosci* **26,** 11938-11947.

Turrigiano G. (2007). Homeostatic signaling: the positive side of negative feedback. *Curr Opin Neurobiol* **17,** 318-324.

Turrigiano G, Abbott LF & Marder E. (1994). Activity-dependent changes in the intrinsic properties of cultured neurons. *Science* **264,** 974-977.

Turrigiano GG. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* **135,** 422-435.

Uchida N & Mainen ZF. (2003). Speed and accuracy of olfactory discrimination in the rat. *Nat Neurosci* **6,** 1224-1229.

Urban NN. (2002). Lateral inhibition in the olfactory bulb and in olfaction. *Physiol Behav* **77,** 607-612.

Urban NN & Sakmann B. (2002). Reciprocal intraglomerular excitation and intra- and interglomerular lateral inhibition between mouse olfactory bulb mitral cells. *The Journal of physiology* **542,** 355-367.

Verhagen JV, Wesson DW, Netoff TI, White JA & Wachowiak M. (2007). Sniffing controls an adaptive filter of sensory input to the olfactory bulb. *Nature neuroscience* **10,** 631-639.

Veruki ML & Hartveit E. (2002). Electrical synapses mediate signal transmission in the rod pathway of the mammalian retina. *J Neurosci* **22,** 10558-10566.

Wachowiak M & Cohen LB. (2001). Representation of odorants by receptor neuron input to the mouse olfactory bulb. *Neuron* **32,** 723-735.

Walmsley B, Alvarez FJ & Fyffe RE. (1998). Diversity of structure and function at mammalian central synapses. *Trends in neurosciences* **21,** 81-88.

Wehr M & Laurent G. (1996). Odour encoding by temporal sequences of firing in oscillating neural assemblies. *Nature* **384,** 162-166.

Wheeler DB, Randall A & Tsien RW. (1996). Changes in action potential duration alter reliance of excitatory synaptic transmission on multiple types of Ca2+ channels in rat hippocampus. *J Neurosci* **16,** 2226-2237.

Wilson DA, Fletcher ML & Sullivan RM. (2004). Acetylcholine and olfactory perceptual learning. *Learn Mem* **11,** 28-34.

Wilson RI & Mainen ZF. (2006). Early events in olfactory processing. *Annu Rev Neurosci* **29,** 163-201.

Wilson RI & Nicoll RA. (2002). Endocannabinoid signaling in the brain. *Science* **296,** 678-682.

Xu J, Kang N, Jiang L, Nedergaard M & Kang J. (2005). Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *J Neurosci* **25,** 1750-1760.

Zalutsky RA & Nicoll RA. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* **248,** 1619-1624.

Zilberter Y, Kaiser KM & Sakmann B. (1999). Dendritic GABA release depresses excitatory transmission between layer 2/3 pyramidal and bitufted neurons in rat neocortex. *Neuron* **24,** 979-988.

Zucker RS. (1973). Changes in the statistics of transmitter release during facilitation. *The Journal of physiology* **229,** 787-810.

Zucker RS & Regehr WG. (2002). Short-term synaptic plasticity. *Annual review of physiology* **64,** 355-405.

Annex: "Glutamatergic transmission and plasticity between olfactory bulb mitral cells"

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Glutamatergic transmission and plasticity between olfactory bulb mitral cells

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In the olfactory bulb the sets of mitral cells that project their apical dendrite to the same glomerulus represent unique functional networks. While it is known that mitral cells release vesicular glutamate from their apical tuft it is believed that the resultant self-excitation (SE), transmitted via dendritic gap junctions, is the main form of lateral transmission within the mitral cell assembly. In this study we used simultaneous whole-cell recordings from mitral cell pairs to show that a direct form of chemical lateral excitation (LE) provides a means of mitral cell–mitral cell communication. In contrast to the ubiquitous expression and robust nature of SE, the efficacy of glutamatergic LE between mitral cells is highly variable and mediated by calcium-impermeable AMPA receptors. We also find that the strength of LE is bi-directionally modulated, in a homeostatic manner, by sniffing-like patterns of presynaptic activity. Since these changes last many minutes we suggest that such mitral cell–mitral cell interactions provide the glomerular network with a locus for olfactory plasticity and a potential mechanism for receptive field modulation.

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Across the surface of the olfactory bulb each glomerulus receives specified inputs from the nose that are integrated by a dedicated ensemble of 50–100 principal mitral and tufted cells. While it is generally agreed that the release of vesicular glutamate from mitral cell dendrites onto local inhibitory interneurons provides a mechanism for recurrent and centre-surround inhibition in the bulb (Pinching & Powell, 1971*a*; Jahr & Nicoll, 1980; Aungst *et al.* 2003; Murphy *et al.* 2005) it is not known how mitral–mitral cell interactions contribute to such processes. Determining whether discharge of an individual mitral cell directly impacts on the excitability of the glomerular mitral cell assembly, will therefore enhance our understanding of the mechanisms of sensory representation within and across such functional modules.

In addition to evoking recurrent and lateral inhibition, the release of glutamate from mitral cell dendrites is also known to evoke both AMPA and NMDA receptor-mediated potentials in the same cell, resulting in a direct form of recurrent self-excitation (SE) (Nicoll & Jahr, 1982; Isaacson, 1999; Margrie *et al.* 2001; Salin *et al.* 2001; Schoppa & Westbrook, 2001, 2002). Simultaneous recordings from intraglomerular pairs of mitral cells also show EPSP-like depolarizations in the second cell (Schoppa & Westbrook, 2002; Urban & Sakmann, 2002; Christie & Westbrook, 2006). Gap junctions located in the tuft, proximal to the source of self excitation (Salin *et al.* 2001) are thought to electrically transmit EPSP-like depolarizations through the mitral cell network (Schoppa & Westbrook, 2002; Christie *et al.* 2005; Christie & Westbrook, 2006). Due to a lack of electron micrographic evidence in support of mitral cell–mitral cell synapses (Price & Powell, 1970; Pinching & Powell, 1971*b*; Kosaka & Kosaka, 2005), lateral transmission is thought to be due solely to gap junction-mediated electrical transfer of SE (Schoppa & Westbrook, 2001, 2002; Christie *et al.* 2005; Christie & Westbrook, 2006). While this form of electrically mediated lateral transmission has been shown to facilitate spike synchronization across the glomerular network (Schoppa & Westbrook, 2001, 2002; Christie *et al.* 2005; Christie & Westbrook, 2006) the precise relationship between SE, electrical coupling and lateral communication between mitral cells is not understood. To date it is assumed that any chemical form of lateral transmission between intraglomerular mitral cells can only occur under conditions that produce non-physiologically elevated levels of glutamate (Schoppa & Westbrook, 2001, 2002; Christie *et al.* 2005; Christie & Westbrook, 2006).

Plasticity within olfactory bulb dendro-dendritic networks has long been proposed to provide a mechanism for olfactory memory (Rosser & Keverne, 1985; Kaba & Keverne, 1988; Sullivan *et al.* 2000), sensory adaptation (Li, 1990), tuning receptive fields and even discrimination learning (Wilson *et al.* 2004). Despite these proposed

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benefits, evidence for neuronal plasticity between dendritic connections in the bulb or for dendritic transmission in general has not been forthcoming. This raises questions as to whether or not dendritic transmission is functionally more hard-wired than axonal transmission and whether or not dendro-dendritic transmission in the olfactory bulb can provide the kind of activity-dependent plasticity observed at many axo-dendritic synapses.

Here we explore directly the relationship between SE, electrical coupling and lateral transmission within intraglomerular mitral cell networks. We show that in addition to electrical coupling, chemical lateral transmission provides a second form of mitral cell–mitral cell communication. The efficacy of this form of dendritic transmission is variable but independent of the strength of electrical coupling and the degree of SE observed in the presynaptic cell. In contrast to SE, glutamatergic lateral excitation (LE) is not ubiquitous and does not rely on calcium-permeable AMPA receptors. Furthermore, and in contrast to electrical transmission, LE provides a dedicated excitatory loop throughout the mitral cell network that is bi-directionally modulated by sniffing-like patterns of mitral cell activity.

Methods

All procedures and animal handling were performed according to the UK Animals (Scientific Procedures) Act 1986. C57BL/6 P20–P27 mice were decapitated and horizontal olfactory bulb slices (300 μm thick) were prepared and maintained in 'low calcium' aCSF (mM: NaCl 125; KCl 2.5; $NaHCO_3$ 26; $NaH_2PO_4.H_2O$ 12.5; $CaCl_2$ 1; $MgCl_2$ 2; glucose 25) equilibrated with 95% O_2–5% CO_2. Whole-cell recordings were performed from the somas of visually identified mitral and tufted cells using infrared differential interference contrast (IR-DIC) microscopy. Electrodes (4–7 MΩ resistance) were filled with a low chloride internal solution containing (mM): methansulphonic acid 130; Hepes 10; KCl 7; EGTA 0.05 Na_2ATP 2; MgATP 2; Na_2GTP 0.5; biocytin 0.4%; titrated to pH 7.4 with 1 M KOH. The extracellular solution contained (mM): NaCl 125; KCl 2.5; $NaHCO_3$ 26; $NaH_2PO_4.H_2O$ 12.5; $CaCl_2$ 2; $MgCl_2$ 1; glucose 26, equilibrated with 95% O_2-5% CO_2 plus 50 μM picrotoxin (unless stated otherwise). Ionotropic glutamate receptor blockers were applied at the following concentrations: AP-V (*R*-2-amino-5-phosphonopentanoate) 25 μM; NBQX (3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*f*)quinoxaline) 10–20 μM; NAS (naphthyl-acethyl-spermine) 20 μM. Whole-cell recordings were obtained using a Multiclamp 700B amplifier, filtered at 6–10 kHz and digitized at 10–20 kHz using an ITC-18 data acquisition board controlled by the Nclamp/Neuromatic package. Data were analysed using Neuromatic (www.neuromatic.thinkrandom.com) software that runs within the Igor Pro (Wavemetrics) environment.

For both SE and LE experiments, action potentials (APs) were evoked by injecting current pulses of 2 ms duration and 1.6–1.8 nA in amplitude. Reported measurements of the strength and monitoring of SE and LE refer to responses evoked by a single AP (except for the quantification of paired-pulse responses). The peak time was determined from an average of at least 12 sweeps and defined as the time at which the membrane voltage reaches the maximum within a 10 ms interval beginning 0.5 ms after the end of the 'presynaptic' current injection. A 2 ms averaging window was centred around the peak time and compared with a 2 ms baseline window that ended 1 ms before the beginning of the presynaptic current injection time. The difference in membrane voltage between the two windows was quantified as being the amount of SE or LE. In cases where LE was smaller than 0.2 mV the peak depolarization values from at least 12 individual sweeps were statistically compared with those from the baseline window. In cases in which the membrane voltage in the second cell was not statistically different from the baseline value, LE was considered to be not detectable. To minimize contamination by voltage-dependent conductances, SE was determined by digitally subtracting the action potential waveforms in the presence of AMPA receptors antagonists from the control waveforms in traces in which the baseline membrane voltage differed by less than 300 μV. Paired-pulse ratios were quantified by dividing the amplitude of the second EPSP by the first, evoked by two APs at 20 Hz. Theta burst stimulation (TBS) consisted of 150 bursts of five APs at 50 Hz repeated at 5 Hz (750 APs, total duration 30 s). Recurrent inhibitory postsynaptic potentials were evoked using a burst of five APs at 50 Hz and determined by digitally subtracting the burst waveforms in the presence of picrotoxin from those under control conditions(Margrie *et al.* 2001). Similarly recurrent IPSP amplitude was quantified as the difference between a 2 ms baseline window positioned before the first AP and a 2 ms detection window which was set centred around the minimum voltage of the subtracted waveform.

Gap junction coupling was assessed by injection of hyperpolarizing pulses (−400 to −800 pA, 600 ms duration) alternating to both cells. Electrical coupling was quantified by determining the ratio (coupling coefficient) between the steady state amplitude of the evoked voltage deflection in the test cell to the response evoked in the presynaptic cell. In cases where the average coupling coefficient for both directions was smaller than 0.01 the postsynaptic hyperpolarizations were checked for statistical significance. In all cases where the morphology of pairs could be confirmed, mitral cells projecting to the same glomerulus showed statistically significant coupling coefficients. The same pulses were used to monitor input resistance during the plasticity experiments.

Dendrotomies were carried out either indirectly (via non-specific amputation during the slicing procedure) or directly using two patch pipettes in a scissor-like motion under visual guidance. In all cases dendritic amputation was confirmed by morphological analysis. Recovery of biocytin-filled cells was carried out as previously described (Horikawa & Armstrong, 1988) and cells were reconstructed using the Neurolucida system (Microbrightfield Inc.). Quantification of mitral cell morphometric properties was performed using the built-in analysis functions of Neurolucida Explorer (integrant part of the Neurolucida System; Microbrightfield Inc.). For the quantification of mitral cell tuft morphology the soma, apical dendrite and lateral dendrites were not considered. For the quantification of lateral dendrites the soma and apical dendrite were excluded. Surface area was computed by treating dentritic segments as frusta. The three-dimensional Convex Hull analysis provides an estimate of the size of the dendritic field of the tufts. It is derived from a convex polygon generated by connecting the tips of the outer dendritic processes. The surface area of the smallest convex polygon for both tufts therefore provides a measure of the space occupied within a glomerulus. For determining the predicted mean surface area for a mitral cell pair, 12 single mitral cells (i.e. cells projecting to different glomeruli, i.e. non-pairs) were reconstructed and their morphologies quantified. Mean values were calculated and multiplied by a factor of two and used as the expected pair morphometry. For the dendritic proximity analysis, histograms reflecting the distance between dendrites belonging to different cells were obtained from Neurolucida Explorer. The minimum distance (or dendritic proximity) was considered to be the lower limit of the first bin (each bin 0.1 μm wide) containing data points.

In several instances displaced mitral cells/tufted cells (whose somas where no more than 166 μm distant from the mitral cell layer) were also recorded from in this study (7 tufted cells out of 198 total cells; no simultaneous recordings from 2 tufted cells are included). Within this subgroup we found representatives of all the functional groups (4 uni-directional, 2 bi-directional and 1 neither direction). We found no significant difference between the amplitude of lateral transmission in tufted cell-containing pairs when compared with the remaining population (tufted cell-containing pairs 0.42 ± 0.26 mV, $n = 6$ connections *versus* exclusively mitral cell pairs 0.53 ± 0.54 mV, $n = 117$connections; $P > 0.05$). Furthermore no significant difference was found in the average tuft morphology (tufted cell-containing pairs: average tuft surface area 6349 ± 2970 μm^2 and 67 ± 36 nodes *versus* exclusively mitral cell pairs 8017 ± 2599 μm^2 and 90 ± 27 nodes; $P > 0.05$). Given these data these cells have been pooled and the general term mitral cell been used. This does not mean to imply that there are not potentially other functional differences between these two cell types (Haberly & Price, 1977; Mori *et al.* 1983; Hayar *et al.* 2004; Nagayama *et al.* 2004; Hayar *et al.* 2005; Karnup *et al.* 2006).

Results

It has been shown that a single action potential in a mitral cell can evoke an EPSP-like depolarization in an intraglomerular partner (Fig. 1*A*, top) that is sensitive to

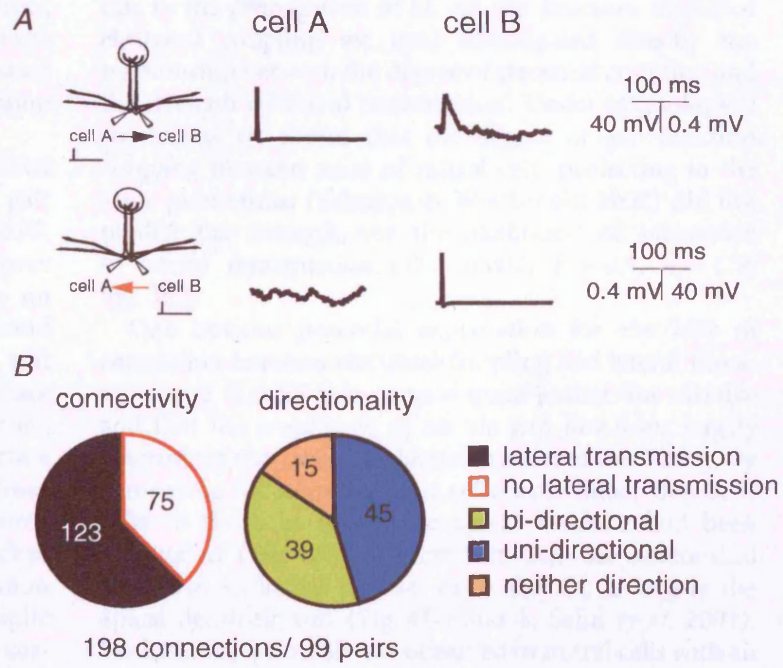

Figure 1. Connectivity and directionality of lateral transmission between mitral cells
A (top), example recordings performed in current clamp from a pair of mitral cells projecting to the same glomerulus. Evoking a single AP in cell A (top left) can result in a detectable depolarization in cell B (top right). For the same pair an AP in cell B failed to evoke detectable lateral transmission (bottom). *B*, pie charts showing the overall connectivity and directionality of lateral transmission across 99 pairs of mitral cells (198 possible connections).

blockers of the AMPA and NMDA subtypes of ionotropic glutamate receptors (Schoppa & Westbrook, 2002; Urban & Sakmann, 2002; Christie & Westbrook, 2006). This phenomenon has been interpreted as lateral transmission of self excitation via gap junctions (Schoppa & Westbrook, 2002). Using simultaneous whole-cell recordings (in the presence of picrotoxin) from pairs of mitral cells (i.e. mitral cells that project their apical dendrites to the same glomerulus; $n = 99$) we find that although lateral transmission is observed in the majority of cases (Fig. 1*B*, top; average amplitude 0.53 ± 0.05 mV $n = 123$ connections) it is not a universal feature of intra-glomerular mitral–mitral cell communication (absent in 75/198 potential conections, Fig. 1*B*). Therefore we sorted pairs into three possible categories according to the expression of lateral transmission: those that expressed lateral transmission in both connections (bi-directional 39/99 pairs; Fig. 1*B* right, green), in a single connection (uni-directional 45/99 pairs; Fig. 1*B* right, blue) or neither of the possible directions (neither direction 15/99 pairs; Fig. 1*B* right, orange). In all the cases where lateral transmission was not observed, the co-localization of mitral cell tufts to the same glomerulus was confirmed either anatomically ($n = 6$) or by the existence of electrical coupling ($n = 9$).

Since the existence and strength of lateral transmission varied considerably between mitral cell pairs one obvious explanation might be that its extent and efficacy varies with intactness of the apical tuft/s which might be compromised by the slicing procedure. We therefore compared the amplitude of lateral transmission between randomly selected pairs ($n = 18$) to several morphometric parameters including the number of branch points, total membrane surface area and the convex Hull surface – a measure of the space occupied by the two dendritic tufts. This morphological data set was then separated into the categories bi-directional, uni-directional or neither, based on the existence and directionality of lateral transmission (Fig. 2*A* and *B*).

Analysis of variance revealed no relationship between the incidence of lateral transmission and the pair tufts macroscopic features (branch points $F_{(2,15)} = 0.97$, $P > 0.4$; tuft surface area $F_{(2,15)} = 0.99$, $P > 0.3$; convex Hull surface $F_{(2,15)} = 0.27$, $P > 0.7$). There was also no correlation between lateral transmission amplitude and the number of branch points ($R^2 = 0.001$, $P > 0.9$), tuft surface area ($R^2 = 0.059$, $P > 0.3$) or convex Hull surface area ($R^2 = 0.003$, $P > 0.7$; Fig. 2*A* and *Ba–c*). Furthermore, the mean number of branch points and membrane surface area of the 18 reconstructed pairs did not differ from morphological data obtained from individual, non-paired mitral cells (Fig. 2*C*; $P > 0.05$). One explanation for lack of correlation between tuft morphometry and the expression and amplitude of lateral transmission could be that, despite being exclusive to intraglomerular pairs, lateral transmission does not occur within the apical tuft but rather via the lateral dendrites. In order to address this notion, we also reconstructed the lateral dendrites of a subset of the previously analysed pairs ($n = 5$) that showed lateral transmission in at least one direction and a mean amplitude identical to that of the recorded population (Fig. 2*Da* and *b*). We found that, in contrast to the tuft where dendritic processes are often in very close opposition (mean minimum distance 0.32 ± 0.07 μm, $n = 5$), lateral dendrites were typically far more distant (closest distance ranging from 1 to 6.5 μm; mean minimum distance 2.98 ± 1.99 μm, $n = 5$). Taken together these data suggest that although the anatomical locus of lateral transmission is likely within the glomerulus, the observed variability in the incidence, directionality and amplitude of lateral transmission does not result from variable amputation of dendritic tufts due to the slicing procedure or naturally occurring morphological differences.

The role of gap junction-mediated coupling and self-excitation in lateral transmission

In no cases have we observed electrical coupling between simultaneously recorded mitral cells whose apical dendritic tufts belonged to different glomeruli (i.e. non-pairs; $n = 14/14$). In contrast, in all cases where gap junction coupling was observed and histological recovery carried out, we observed a perfect correspondence between coupling and the glomerular location of the dendritic tufts (Fig. 3*A* and *B*; $n = 133$; Schoppa & Westbrook, 2002; Christie *et al.* 2005; Christie & Westbrook, 2006). Since lateral transmission between mitral cells is proposed to be due to the propagation of SE via gap junction-mediated electrical coupling we next investigated directly the relationship between the degree of electrical coupling and the strength of lateral transmission. Under physiological conditions we found that the degree of gap junction coupling between pairs of mitral cells projecting to the same glomerulus (Schoppa & Westbrook, 2002) did not predict the strength nor the likelihood of expression of lateral transmission ($R^2 = 0.002$, $P > 0.5$, $n = 128$; Fig. 3*C*).

One obvious potential explanation for the lack of correlation between electrical coupling and lateral transmission is that SE is in general quite potent but variable and that the amplitude of SE via gap junctions largely determines the extent of lateral transmission. Firstly, by comparing SE amplitude in cells with intact dendritic tufts to those in which the apical dendrite had been amputated (Fig. 4*A*) it is evident that the anatomical locus for SE in the mouse, as in the rat, is largely the apical dendritic tuft (Fig. 4*Ba* and *b*; Salin *et al.* 2001). Without exception SE was observed in mitral cells with an

intact tuft, ranging in amplitude from 0.62 mV to 8.5 mV (average 2.04 ± 0.31 mV, 28/28 cells; Fig. 4*Ba* left and *Bb*, filled bars). In contrast, all recorded dendrotomised cells lacked SE (average 0.03 ± 0.05 mV, $n = 5/5$; Fig. 4*Ba* right, and Fig. 4*Bb*, open bar). One further possibility, however, is that dendrotomy leads to a general degradation in the release machinery within apical and lateral dendrites. To assess this possibility we also investigated the effect of

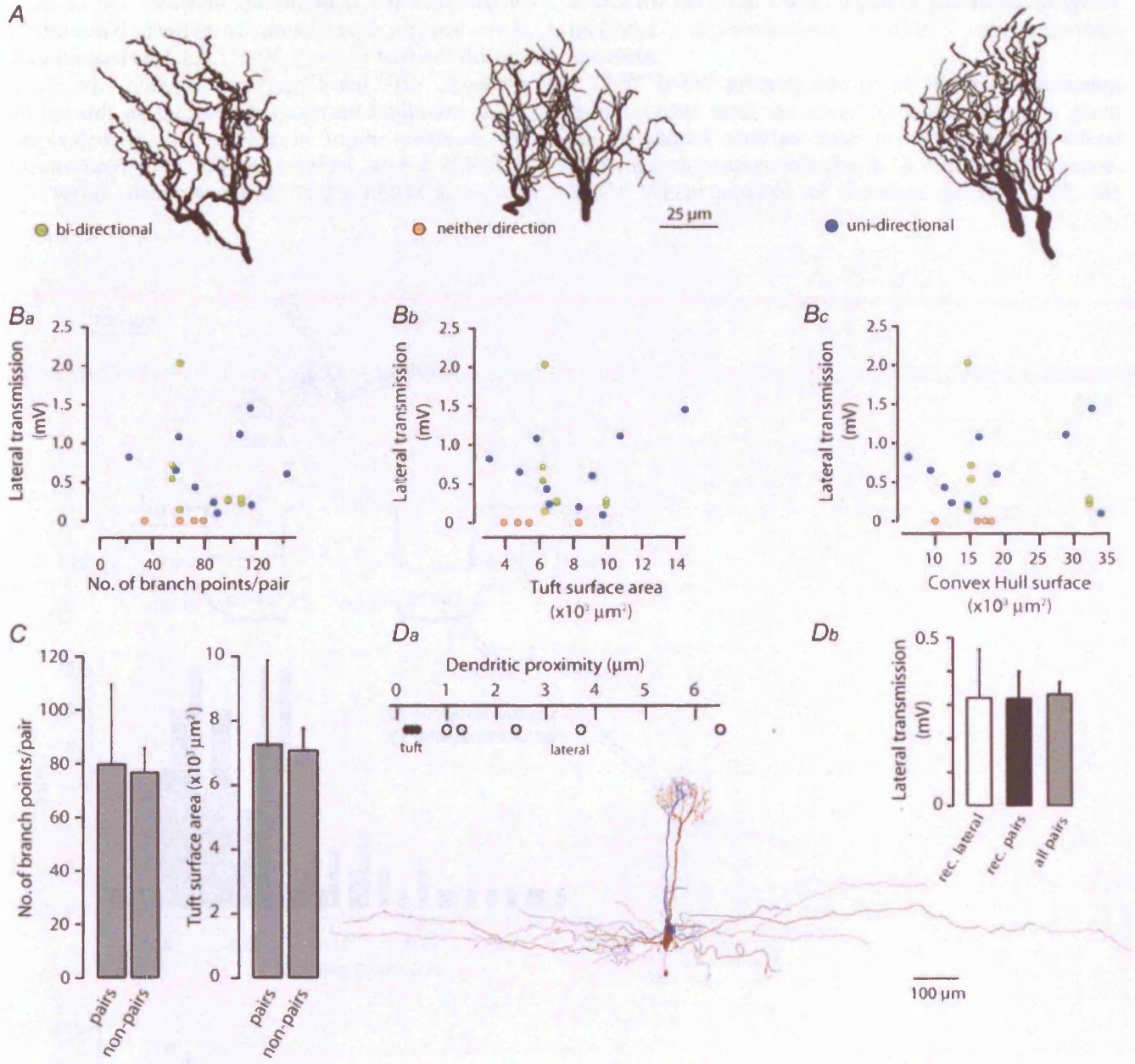

Figure 2. The efficacy and occurrence of lateral transmission does not correlate with tuft morphology
A, example Neurolucida reconstructions of pairs of dendritic tufts for each of the three lateral transmission directional groupings. *B*, graphs showing the relationship between the total number of branch points (*Ba*), surface area (*Bb*) and convex Hull surface (*Bc*) and the amplitude of lateral transmission (bi-directional (both directions plotted), $n = 8$ connections (green circles); uni-directional (a single direction plotted), $n = 10$ (blue circles); neither direction (a single direction plotted), $n = 4$ (orange circles); total, $n = 22$ connections from 18 pairs). *C*, comparison of the average morphometric properties of both reconstructed tufts per pair ($n = 18$ pairs) to those from individual cells that did not project their apical dendrites to the same glomerulus (non-pairs; $n = 12$ cells, each tuft multiplied by a factor of 2; error bars correspond to standard deviation). *D*, in a subset of pairs lateral dendrites ($n = 5$) were also reconstructed (inset bottom). *Da*, a plot showing the minimum distance bin (bins were 0.1 µm wide; lower bin limit plotted – see Methods) obtained from a histogram of dendritic distances (●, apical tuft; ○, lateral dendrites). *Db*, a plot showing the amplitude of lateral transmission for pairs where lateral dendrites were reconstructed (rec. laterals, $n = 5$ pairs), all pairs where the tufts were reconstructed (rec. pairs $n = 18$ pairs) and for the entire data set (all pairs, $n = 99$ pairs; bars correspond to standard error).

dendrotomy on the recurrent IPSP that, at least in part, will be evoked by glutamate release from mitral cell lateral dendrites (Rall *et al.* 1966; Jahr & Nicoll, 1982; Margrie *et al.* 2001). We find that, in contrast to SE, recurrent inhibition is not impaired in dendrotomised cells (Fig. 4*C*, intact 2.46 ± 1.28 mV $n = 5$; dendrotomised 6.52 ± 6.11 mV, $P > 0.05$ Mann–Whitney test; $n = 5$). Though not significant, the apparent trend towards an increase in recurrent inhibition could be explained by an increase in input resistance for dendrotomised cells (Fig. 4*C*, intact 47.4 ± 20.3 MΩ, $n = 7$ *versus* dendrotomised 75.8 ± 17.0 MΩ, $n = 7$, $P < 0.05$ Mann–Whitney test; (Bekkers & Hausser, 2007). Taken together, our dendrotomy data indicate that SE is a universal feature of mitral cell excitability and occurs primarily in the apical tuft, providing an ideal source for electrical transmission of glutamate receptor-mediated depolarizations within intraglomerular networks.

If SE is the primary source of lateral transmission then together with the coupling coefficient of a given pair it should correlate with the amplitude of lateral transmission between mitral cells (Schoppa & Westbrook, 2002). Where possible we therefore quantified SE, the

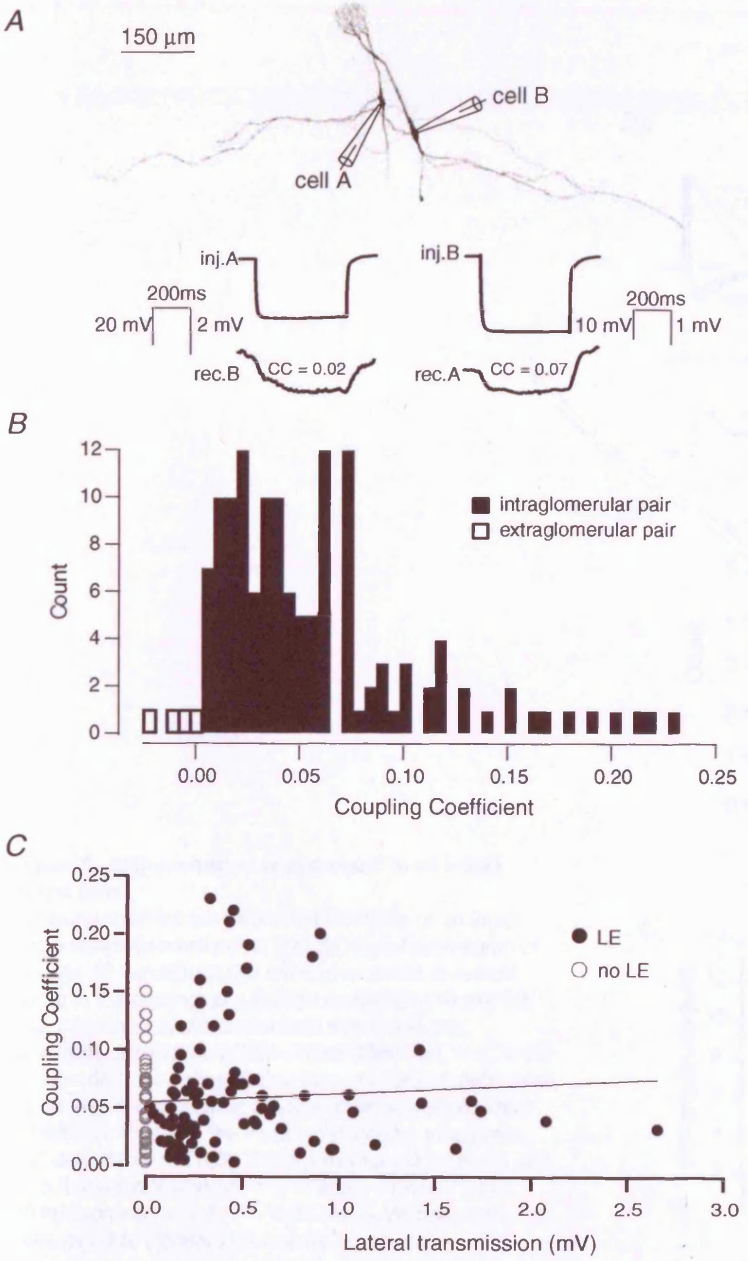

Figure 3. Electrical coupling is ubiquitous and reciprocal among intraglomerular mitral cells
A (top), example reconstructions of a morphologicaly defined pair of mitral cells. Bottom, example average traces (20 sweeps) recorded in current clamp showing bi-directional electrical coupling assessed by hyperpolarizing pulses in each cell. *B*, histogram showing the distribution of coupling coefficients for 133 connections (filled bars) and 4 non-pairs (open bars). *C*, plot showing the lack of correlation between electrical coupling and lateral transmission.

coupling coefficients and lateral transmission for the same mitral–mitral cell connections ($n = 10$; Fig. 5). While some connections showed a modest amount of SE and coupling with robust lateral transmission, others showed small or no detectable lateral transmission despite similar degrees of coupling and moderate SE. Overall we found no correlation between SE, the degree of coupling and lateral transmission (Fig. 5, $R^2 = 0.11$ $P > 0.3$, $n = 10$). This lack of correlation hints at the existence of an alternative form of transmission between mitral cells.

Self-excitation and lateral transmission are distinct forms of mitral cell signalling

Recent studies indicate that both calcium-permeable and calcium-impermeable AMPA receptors co-exist within

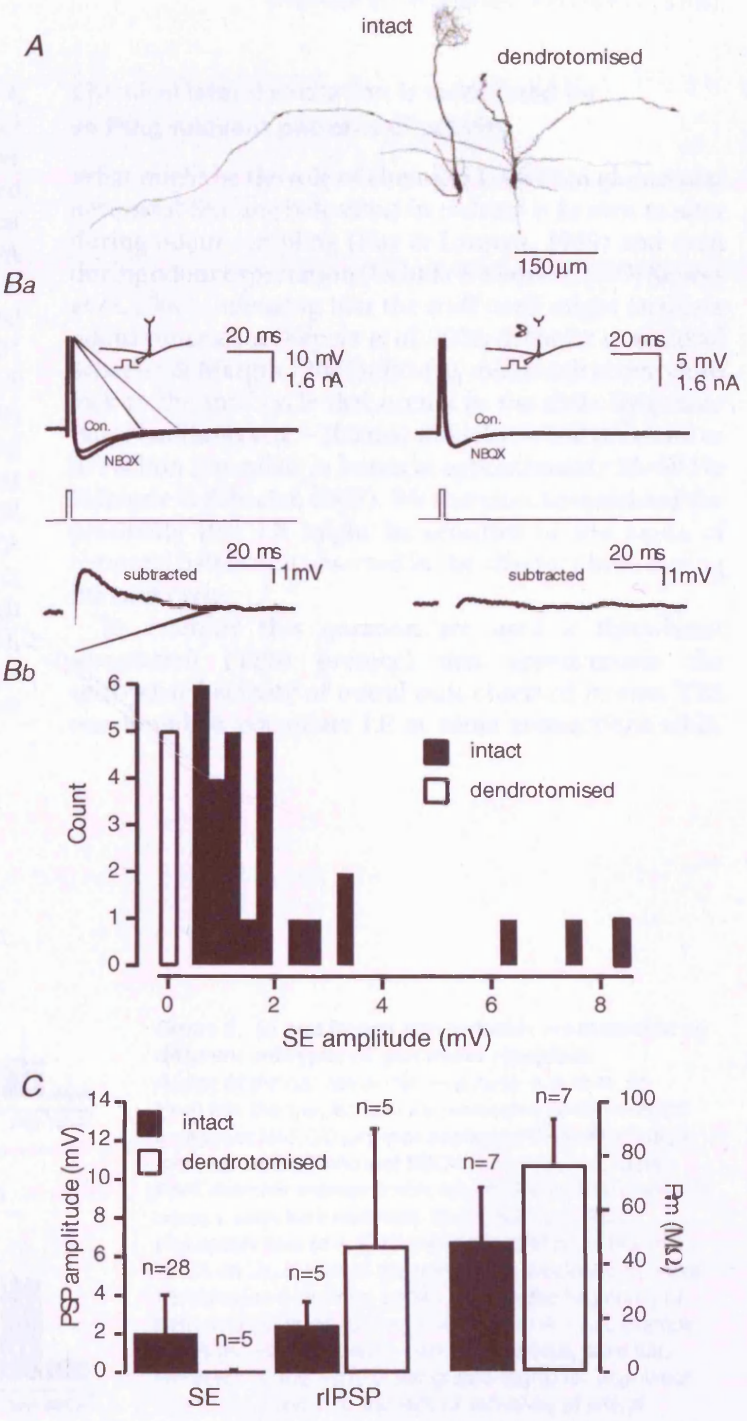

Figure 4. Self-excitation is expressed in all intact mitral cells
A, Neurolucida reconstructions of examples of an intact and a dendrotomised mitral cell. *Ba*, digital subtraction of average AP waveforms (30 sweeps) recorded in current clamp in the absence and presence of NBQX (10 μM) for intact and apically dendrotomised mitral cells. *Bb*, amplitude distribution of SE in intact (filled bars, $n = 28/28$) and dendrotomised cells (open bars, $n = 5/5$). *C* (left), plot comparing the amplitude of self-excitation and recurrent inhibition (rIPSP) and the input resistance for intact cells and dendrotomised cells. Resting membrane potential was -55.0 ± 4.9 mV (control, $n = 5$) and -51.4 ± 3.0 mV (dendrotomised, $n = 5$; $P > 0.05$ Mann–Whitney test; bars correspond to standard deviation).

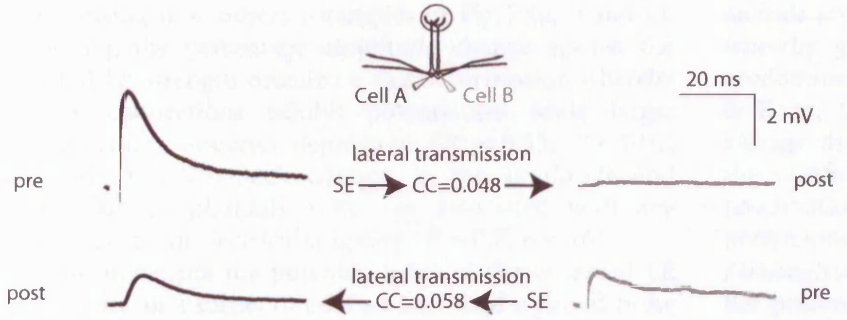

Figure 5. Expression of self excitation and lateral transmission
Example traces (15 sweeps) from a simultaneously recorded pair of cells in which SE, the coupling coefficient and lateral transmission were determined for both directions. In one direction (cell A→cell B; top) large SE (3.60 mV) was observed which resulted in modest lateral transmission (0.15 mV). In contrast, in the other direction (cell B→cell A; bottom) we observed lateral transmission (1.05 mV) that was similar in amplitude to the observed SE in cell B (1.13 mV).

olfactory bulb glomeruli (Montague & Greer, 1999; Blakemore *et al.* 2006; Ma & Lowe, 2007). In order to potentially dissect SE and lateral transmission we examined whether SE and lateral transmission relied on different types of AMPA receptors. We found that bath application of the specific calcium-permeable AMPA receptor antagonist naphthyl-acethyl-spermine (NAS, 20 μM) blocked SE by approximately 71% (residual was 28.6 ± 21.3% of control amplitude $n = 8$, $P < 0.01$ Wilcoxon paired sample test; Fig. 6*A*). In contrast NAS had little effect on lateral transmission (80.8 ± 21.4% of control amplitude $n = 7$, $P > 0.05$ Wilcoxon paired sample test; Fig. 6*B*). These experiments confirm that SE within and lateral transmission between mitral cells are predominantly independent forms of AMPA receptor-mediated excitation. The existence of direct glutamatergic transmission between mitral cells (which we will now refer to as chemical lateral excitation, LE), suggests that it might serve a functional role in glomerular processing rather then being an epiphenomenon of SE.

Chemical lateral excitation is modulated by sniffing-relevant patterns of activity

What might be the role of chemical LE within glomerular networks? Sniffing behaviour in rodents is known to alter during odour sampling (Kay & Laurent, 1999) and even during odour expectation (Uchida & Mainen, 2003; Kepecs *et al.* 2006) indicating that the sniff itself might facilitate odour processing (Kepecs *et al.* 2006; Schaefer *et al.* 2006; Schaefer & Margrie, 2007). *In vivo*, mitral cell activity can lock to the sniff cycle that occurs in the theta frequency range (sniffing cycle ~200 ms) whereby mitral cells tend to fire action potentials in bursts at approximately 50–60 Hz (Margrie & Schaefer, 2003). We therefore investigated the possibility that LE might be sensitive to the kinds of temporal patterning observed in the olfactory bulb during the sniff cycle.

To examine this question we used a theta-burst stimulation (TBS) protocol that approximates the sniff-related activity of mitral cells observed *in vivo*. TBS was found to potentiate LE at some connections while

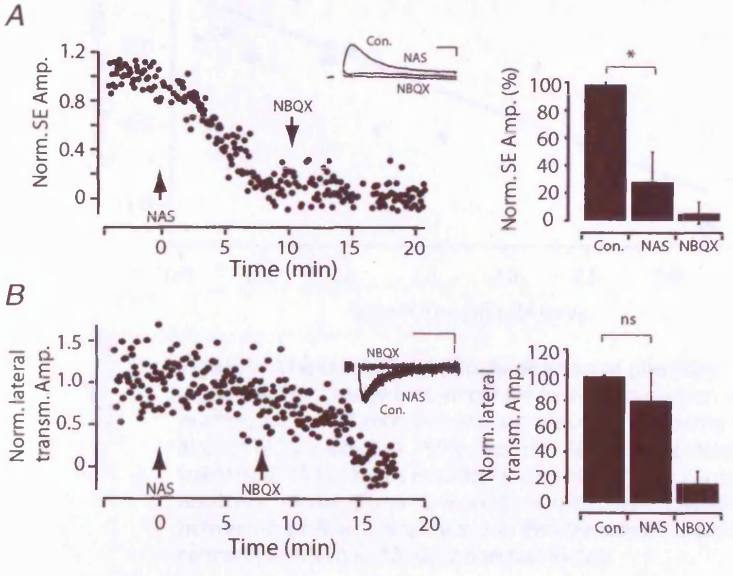

Figure 6. SE and lateral transmission are mediated by different subtypes of glutamate receptors
A, plot of the normalized SE amplitude over time. At $t = 0$ min the specific calcium-permeable AMPA receptor antagonist NAS (20 μM) was applied to the bath solution followed by application of NBQX (20 μM) at $t = 10$ min. Inset, example average traces recorded in current clamp (30 sweeps, scale bars represent 10 ms, 500 μV). Right, population data ($n = 8$) showing the effects of NAS and NBQX on SE. *B*, plot of the normalized amplitude of lateral transmission over time; arrows indicate the beginning of bath application of NAS and NBQX as in A. Inset, example traces recorded in voltage clamp (30 sweeps, scale bars represent 10 ms, 10 pA). Bar graphs (right) for population data ($n = 7$) showing the lack of sensitivity of lateral transmission to NAS (bars correspond to standard deviation).

depressing it at others (examples in Fig. 7*Aa*, *b* and *c*). Plotting the percentage amplitude change against the initial LE strength revealed a linear correlation whereby weak connections exhibit potentiation while larger connections undergo depression ($R^2 = 0.53$, $P < 0.01$; $n = 18$; Fig. 7*B*). Such changes in the amplitude and direction of plasticity were not associated with any modulation of electrical coupling ($P > 0.7$, $n = 14$).

To investigate the potential locus of the observed LE plasticity, in a subset of cells we first used a paired-pulse protocol that assays potential changes in the probability of transmitter release (Fatt & Katz, 1952; Zucker & Regehr, 2002). In all cases where this was examined TBS-induced changes in LE strength parallelled changes in the paired-pulse ratio ($R^2 = 0.91$, $P < 0.05$, Fig. 8*A*, $n = 5/5$) whereby increases in LE efficacy were associated with a decrease in the paired-pulse facilitation ratio and vice versa. To further assess the possibility that presynaptic mechanisms may be involved in LE plasticity, we determined whether a change in the coefficient of variation of LE also parallels the direction and amplitude of plasticity. Normalized CV^2/amplitude plots can provide an indication of the locus of changes in synaptic efficacy whereby gradients steeper than unity are considered predominantly presynaptic (Malinow & Tsien, 1990; Faber & Korn, 1991; Hardingham *et al.* 2007). We find on average that the gradient of the ratiometric change in the coefficient of variation for both depression and potentiation is steeper than the unity line indicating a presynaptic component (Fig. 8*Ba* and *b*, respectively). Although several connections fell close to and some outside the presynaptic locus quadrant (see Fig. 8*B*) such cells often displayed large changes in EPSP variance in the predicted direction (for example see Fig. 7*Ab* and *c*) but rather the amplitude of the EPSP appeared to change to a lesser extent (example Figs 7*Ab* and *c*, and 8*Ba* and *b*). This together with our paired-pulse data suggest that a presynaptic mechanism is probably involved in LE plasticity within mitral cell assemblies.

Discussion

Here we show that within the glomerulus network under physiological conditions mitral cells communicate via

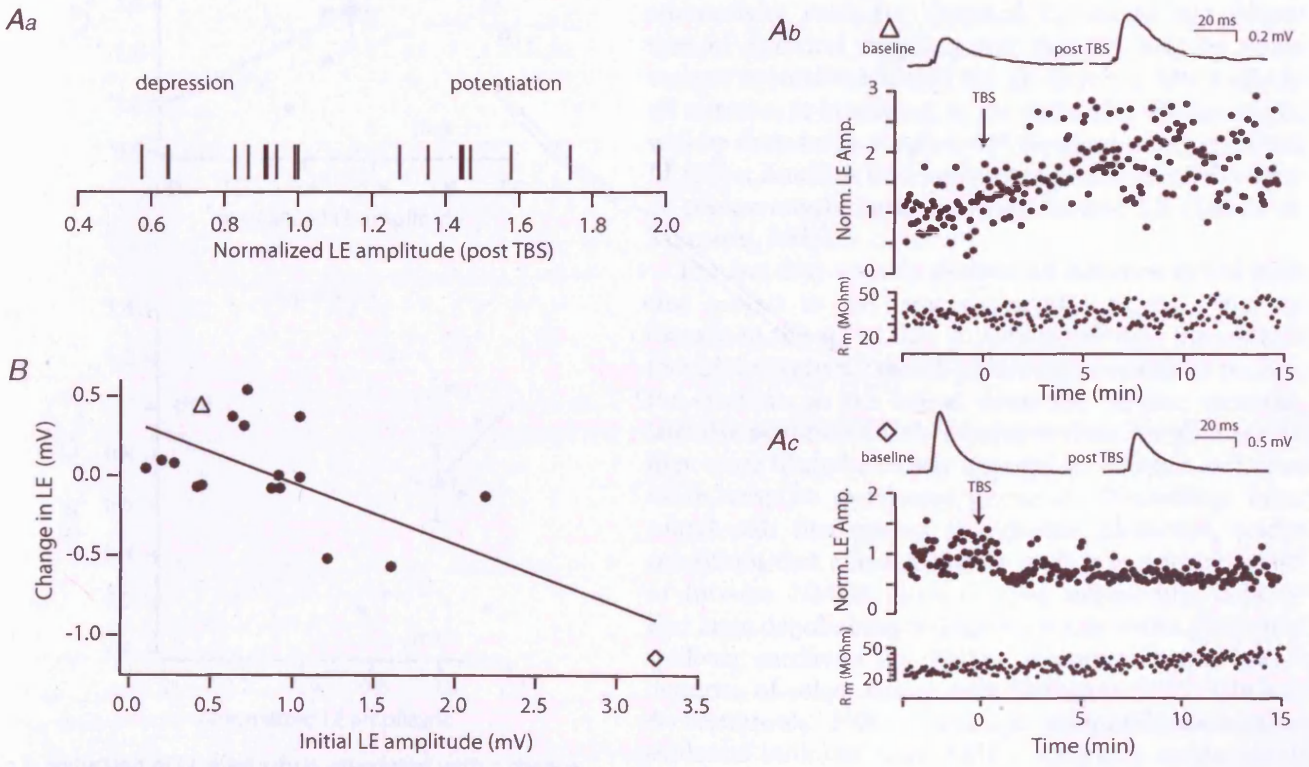

Figure 7. Chemical LE exhibits bi-directional plasticity
Aa, normalized change in LE amplitude for each connection. *Ab* and *Ac*, plots of LE amplitude over time illustrating examples of potentiation (*Ab*) and depression (*Ac*) following theta-burst stimulation (onset of TBS indicated by the arrow). TBS consisted of 150 bursts of 5 APs at 50 Hz repeated at 5 Hz (750 APs, total duration 30 s). Average traces (top) of 30 sweeps recorded in current clamp during the baseline and following TBS. Below is a plot of input resistance measured over time using a somatic hyperpolarizing pulse. *B*, population data showing the relationship between the initial LE amplitude and the observed absolute change in amplitude ($R^2 = 0.53$, $P < 0.01$ $n = 18$; △, connection shown in *Ab*; ◇, connection in *Ac*).

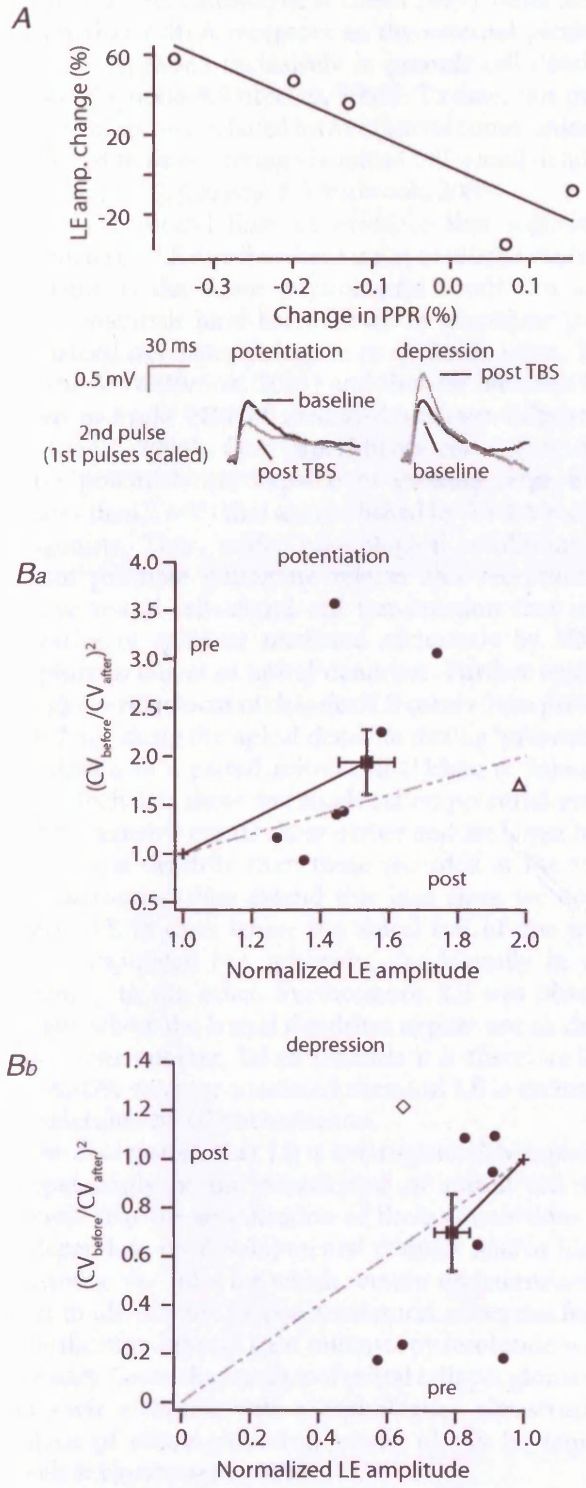

Figure 8. Induction of LE plasticity is associated with a change in dendritic release

A, a plot of the change in paired-pulse ratio (PPR) as it relates to changes in LE induced by TBS. Pairs of APs delivered at 20 Hz were used to evoke EPSPs in the second cell. The amplitude of the second EPSP was divided by the first and normalized over the baseline period (data from 5/5 pairs in which PPR was monitored). Below, average traces of the second EPSP (30 sweeps) recorded during the baseline period and following potentiation (left) and depression (right). The amplitude of the first EPSP is scaled (not shown) to highlight the change in PPR. *B*, the change in the coefficient of variation (CV) following TBS plotted against post-TBS LE amplitude for potentiation (*Ba*) and depression (*Bb*) (△, connection shown in Fig. 7*Ab*; ◇, connection in Fig. 7*Ac*; bars represent standard error).

electrical and chemical transmission. Our data show that while self-excitation and electrical coupling are robust and reliable hallmarks of mitral cells, together they do not account for the reliability and strength of lateral transmission.

The most direct evidence that LE is an independent form of mitral cell communication comes from the observation that SE and LE rely on the activation of different types of AMPA receptors. While SE is mediated by calcium-permeable AMPA receptors, LE shows little sensitivity to a specific antagonist (NAS) for these receptors. Recently, calcium-permeable AMPA receptors have been shown to be crucial in some forms of synaptic plasticity (Cull-Candy *et al.* 2006) and in the retina, where expressed presynaptically, they can mediate transmitter release (Chavez *et al.* 2006). The fact that LE was found to be largely insensitive to NAS suggests that calcium-permeable AMPA receptors here are involved neither in LE release nor reception within intraglomerular mitral cell dendrites.

Our morphometric analysis of mitral cell pairs indicates that macroscopic features of tuft anatomy do not explain the likelihood of LE between a given cell pair. This may be indicative of several features of LE. Firstly, the connectivity rules for chemical LE might not follow that of electrical coupling and that LE may be more compartmentalized within the glomerulus. Alternatively all mitral cells belonging to the same glomerulus might well be chemically coupled and the cases of no apparent LE reflect dendritic filtering by the tuft and apical dendrite of comparatively small or more distant LE (Urban & Sakmann, 2002).

The fact that we only observe LE between mitral cells that project to the same glomerulus suggests that its locus is in the apical tuft. A third possibility, however, is that LE between mitral cell glomerular assemblies occurs, for example, in the lateral dendrites. In this scenario, lateral dendrites of cells belonging to the same glomerulus may more likely be closely apposed to another and thus more receptive to released glutamate. Recordings from mitral cells that project to different glomeruli, under conditions that enhance release and/or prevent reuptake or increase NMDA channel open probability, indicate that large depolarizing voltage steps can evoke glutamate spillover mediated by NMDA receptors in the lateral dendrite of other mitral cells (Isaacson, 1999; Christie & Westbrook, 2006). Although immunohistochemical evidence indicates that AMPA receptors reside along

lateral dendrites (Montague & Greer, 1999), other studies suggest that AMPA receptors in the external plexiform layer are expressed exclusively in granule cell dendrites (Sassoe-Pognetto & Ottersen, 2000). To date, this purely NMDA receptor-mediated form of lateral communication is believed to be occurring via mitral cell lateral dendrites (Isaacson, 1999; Christie & Westbrook, 2006).

We have several lines of evidence that suggest the glutamatergic LE decribed here is not mediated via lateral dendrites. Under more physiological conditions single action potentials have been shown to propagate poorly into lateral dendrites (Margrie *et al.* 2001; Lowe, 2002; Christie & Westbrook, 2003) and thus far have not been shown to evoke NMDA-mediated spillover (Christie & Westbrook, 2006). Our experiments show that single action potentials are capable of evoking large EPSPs (greater than 2 mV) that are abolished by AMPA receptor antagonists. Thus, under physiological conditions that do not promote glutamate release and reception, we observe mitral cell–mitral cell transmission that is not indicative of spillover mediated exclusively by NMDA receptors in lateral or apical dendrites. Further evidence for a glomerular locus of chemical LE comes from previous recordings along the apical dendrite during 'presynaptic' stimulation of a paired mitral cell (Urban & Sakmann, 2002). Such data show that single action potential-evoked AMPA-mediated events occur earlier and are larger in the distal apical dendrite than those recorded at the soma. Our anatomical data extend this idea since we do not observe LE in cases where the apical tuft of one mitral cell is amputated but otherwise dendritically in close proximity to the other. Furthermore, LE was observed in pairs where the lateral dendrites appear not to closely oppose one another. Taken together it is therefore likely that AMPA receptor-mediated chemical LE is exclusively a glomerulus-based phenomenon.

The observation that LE is heterogeneously expressed, independently of the overall size of mitral cell tufts, suggests that the specification of these connections may be dependent on developmental features and/or history of activity, the rules for which remain undetermined. In order to identify the precise anatomical substrates for LE, quantification beyond light microscopy resolution will be necessary. Given the number of mitral cells per glomerulus and their elaborate tuft morphologies, ultrastructural analysis of entire glomeruli would ideally be required (Denk & Horstmann, 2004).

To date lateral transmission between mitral cells has previously been suggested to rely solely on gap junction-mediated transfer of SE (Schoppa & Westbrook, 2002; Christie *et al.* 2005). The direct evidence for this comes from a lack of lateral transmission observed in connexin36 knockout mice (Schoppa & Westbrook, 2002; Christie *et al.* 2005; Christie & Westbrook, 2006). Rather than a knockout approach we have used a correlation-based approach to examine any relation between SE, electrical coupling and LE. Together these data suggest that while gap junction-mediated coupling may be a requirement for the development of chemical LE, the degree of coupling does not determine the efficacy of LE. Such studies in connexin36 knockout mice further show that chemical transmission between mitral cell pairs can only occur during periods of high frequency activity in the presence of glutamate uptake blockers (Christie & Westbrook, 2006). In contrast, we find that under physiological conditions LE can be evoked by a single action potential, does not require artificially high concentrations of glutamate, and is therefore likely to contribute to intraglomerular processing.

Gap junctions provide cells with electrical coupling via membrane pores that allow the flux of ions and even metabolites. Ionic flow is linear, bi-directional and can be of either polarity (Sohl *et al.* 2005). In mitral cells, depending on the time course and membrane potential, it is expected that inhibition, excitatory sensory drive and SE will propagate equally efficiently through such electrical synapses. This way electrical coupling could co-ordinate excitation and inhibition across connected cells, facilitating the synchronization of activity within the entire assembly (Bennett & Zukin, 2004). In contrast to gap junctions that do not discriminate in their transmission, chemical LE provides an exclusive excitatory pathway within the mitral cell network. Despite the fact that LE evoked by a single AP is typically modest in amplitude when measured at the soma, when evoked by several synchronous mitral cells it will substantially depolarize the postsynaptic cell. This is even more probable when considering that large-amplitude subthreshold oscillations are likely to further co-ordinate mitral activity around very depolarized membrane potentials *in vivo* (Luo & Katz, 2001; Cang & Isaacson, 2003; Margrie & Schaefer, 2003; Schaefer *et al.* 2006).

It is well known that dendritic release can be modulated by neuromodulators (Isaacson & Vitten, 2003; Davison *et al.* 2004) and high-frequency bursts of activity (Wilson & Nicoll, 2002; Freund *et al.* 2003). Here we show that in mitral cells, bursts of action potentials in the theta frequency range can regulate intraglomerular dendritic transmission. The sensitivity of LE to sniffing-like patterns of activity suggests that the efficacy of LE will be modulated under physiological conditions *in vivo* (Margrie & Schaefer, 2003; Schaefer *et al.* 2006; Schaefer & Margrie, 2007; Verhagen *et al.* 2007). In slices, theta-burst stimulation could induce changes in LE efficacy such that small connections were potentiated while larger connections showed depression. Recent work in layer 2/3 neocortical pyramidal cells show similarly that the initial strength of axo-dendritic connections can determine the direction and the amount of change in efficacy in a manner that is dependent on release probability (Hardingham

et al. 2007). Consistent with these findings, we observe that the direction and amplitude of plasticity correlates with changes in the paired-pulse ratio. As with layer 2/3 neurons, changes in the CV before and after induction of plasticity further indicate a presynaptic contribution.

To date the major form of plasticity within the bulb relies on neurogenesis whereby new interneurons are continuously integrated into the bulb circuitry over the time course of several weeks (Lledo *et al.* 2006). Our data show that instantaneous forms of dendro-dendritic plasticity also exist. One idea is that LE could provide a mechanism to change the gain function of an individual cell to match the overall glomerular input. This may act to compensate for flux in the ongoing degeneration and genesis of the sensory input that an individual mitral cell receives. In this manner, LE plasticity could serve to normalize sensitivity and provide reliable sensory reception and transmission at the level of the intra-glomerular mitral cell network. Such network homeostasis mechanisms would ensure that output from those cells belonging to the same functional assembly provide similar output patterns to different target cell populations in downstream structures (Suzuki & Bekkers, 2006).

References

Aungst JL, Heyward PM, Puche AC, Karnup SV, Hayar A, Szabo G & Shipley MT (2003). Centre-surround inhibition among olfactory bulb glomeruli. *Nature* **426**, 623–629.

Bekkers JM & Hausser M (2007). Targeted dendrotomy reveals active and passive contributions of the dendritic tree to synaptic integration and neuronal output. *Proc Natl Acad Sci U S A* **104**, 11447–11452.

Bennett MV & Zukin RS (2004). Electrical coupling and neuronal synchronization in the mammalian brain. *Neuron* **41**, 495–511.

Blakemore LJ, Resasco M, Mercado MA & Trombley PQ (2006). Evidence for Ca^{2+}-permeable AMPA receptors in the olfactory bulb. *Am J Physiol Cell Physiol* **290**, C925–C935.

Cang J & Isaacson JS (2003). In vivo whole-cell recording of odor-evoked synaptic transmission in the rat olfactory bulb. *J Neurosci* **23**, 4108–4116.

Chavez AE, Singer JH & Diamond JS (2006). Fast neurotransmitter release triggered by Ca influx through AMPA-type glutamate receptors. *Nature* **443**, 705–708.

Christie JM, Bark C, Hormuzdi SG, Helbig I, Monyer H & Westbrook GL (2005). Connexin36 mediates spike synchrony in olfactory bulb glomeruli. *Neuron* **46**, 761–772.

Christie JM & Westbrook GL (2003). Regulation of backpropagating action potentials in mitral cell lateral dendrites by A-type potassium currents. *J Neurophysiol* **89**, 2466–2472.

Christie JM & Westbrook GL (2006). Lateral excitation within the olfactory bulb. *J Neurosci* **26**, 2269–2277.

Cull-Candy S, Kelly L & Farrant M (2006). Regulation of Ca^{2+}-permeable AMPA receptors: synaptic plasticity and beyond. *Curr Opin Neurobiol* **16**, 288–297.

Davison IG, Boyd JD & Delaney KR (2004). Dopamine inhibits mitral/tufted → granule cell synapses in the frog olfactory bulb. *J Neurosci* **24**, 8057–8067.

Denk W & Horstmann H (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biol* **2**, e329.

Faber DS & Korn H (1991). Applicability of the coefficient of variation method for analyzing synaptic plasticity. *Biophys J* **60**, 1288–1294.

Fatt P & Katz B (1952). Spontaneous subthreshold activity at motor nerve endings. *J Physiol* **117**, 109–128.

Freund TF, Katona I & Piomelli D (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* **83**, 1017–1066.

Haberly LB & Price JL (1977). The axonal projection patterns of the mitral and tufted cells of the olfactory bulb in the rat. *Brain Res* **129**, 152–157.

Hardingham NR, Hardingham GE, Fox KD & Jack JJ (2007). Presynaptic efficacy directs normalization of synaptic strength in layer 2/3 rat neocortex after paired activity. *J Neurophysiol* **97**, 2965–2975.

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

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

Horikawa K & Armstrong WE (1988). A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. *J Neurosci Meth* **25**, 1–11.

Isaacson JS (1999). Glutamate spillover mediates excitatory transmission in the rat olfactory bulb. *Neuron* **23**, 377–384.

Isaacson JS & Vitten H (2003). $GABA_B$ receptors inhibit dendrodendritic transmission in the rat olfactory bulb. *J Neurosci* **23**, 2032–2039.

Jahr CE & Nicoll RA (1980). Dendrodendritic inhibition: demonstration with intracellular recording. *Science* **207**, 1473–1475.

Jahr CE & Nicoll RA (1982). An intracellular analysis of dendrodendritic inhibition in the turtle *in vitro* olfactory bulb. *J Physiol* **326**, 213–234.

Kaba H & Keverne EB (1988). The effect of microinfusions of drugs into the accessory olfactory bulb on the olfactory block to pregnancy. *Neuroscience* **25**, 1007–1011.

Karnup SV, Hayar A, Shipley MT & Kurnikova MG (2006). Spontaneous field potentials in the glomeruli of the olfactory bulb: the leading role of juxtaglomerular cells. *Neuroscience* **142**, 203–221.

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

Kepecs A, Uchida N & Mainen ZF (2006). The sniff as a unit of olfactory processing. *Chem Senses* **31**, 167–179.

Kosaka T & Kosaka K (2005). Intraglomerular dendritic link connected by gap junctions and chemical synapses in the mouse main olfactory bulb: electron microscopic serial section analyses. *Neuroscience* **131**, 611–625.

Li Z (1990). A model of olfactory adaptation and sensitivity enhancement in the olfactory bulb. *Biol Cybern* **62**, 349–361.

Lledo PM, Alonso M & Grubb MS (2006). Adult neurogenesis and functional plasticity in neuronal circuits. *Nat Rev Neurosci* **7**, 179–193.

Lowe G (2002). Inhibition of backpropagating action potentials in mitral cell secondary dendrites. *J Neurophysiol* **88**, 64–85.

Luo M & Katz LC (2001). Response correlation maps of neurons in the mammalian olfactory bulb. *Neuron* **32**, 1165–1179.

Ma J & Lowe G (2007). Calcium permeable AMPA receptors and autoreceptors in external tufted cells of rat olfactory bulb. *Neuroscience* **144**, 1094–1108.

Malinow R & Tsien RW (1990). Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* **346**, 177–180.

Margrie TW, Sakmann B & Urban NN (2001). Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb. *Proc Natl Acad Sci U S A* **98**, 319–324.

Margrie TW & Schaefer AT (2003). Theta oscillation coupled spike latencies yield computational vigour in a mammalian sensory system. *J Physiol* **546**, 363–374.

Montague AA & Greer CA (1999). Differential distribution of ionotropic glutamate receptor subunits in the rat olfactory bulb. *J Comp Neurol* **405**, 233–246.

Mori K, Kishi K & Ojima H (1983). Distribution of dendrites of mitral, displaced mitral, tufted, and granule cells in the rabbit olfactory bulb. *J Comp Neurol* **219**, 339–355.

Murphy GJ, Darcy DP & Isaacson JS (2005). Intraglomerular inhibition: signaling mechanisms of an olfactory microcircuit. *Nat Neurosci* **8**, 354–364.

Nagayama S, Takahashi YK, Yoshihara Y & Mori K (2004). Mitral and tufted cells differ in the decoding manner of odor maps in the rat olfactory bulb. *J Neurophysiol* **91**, 2532–2540.

Nicoll RA & Jahr CE (1982). Self-excitation of olfactory bulb neurones. *Nature* **296**, 441–444.

Pinching AJ & Powell TP (1971*a*). The neuron types of the glomerular layer of the olfactory bulb. *J Cell Sci* **9**, 305–345.

Pinching AJ & Powell TP (1971*b*). The neuropil of the glomeruli of the olfactory bulb. *J Cell Sci* **9**, 347–377.

Price JL & Powell TP (1970). The mitral and short axon cells of the olfactory bulb. *J Cell Sci* **7**, 631–651.

Rall W, Shepherd GM, Reese TS & Brightman MW (1966). Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Exp Neurol* **14**, 44–56.

Rosser AE & Keverne EB (1985). The importance of central noradrenergic neurones in the formation of an olfactory memory in the prevention of pregnancy block. *Neuroscience* **15**, 1141–1147.

Salin PA, Lledo PM, Vincent JD & Charpak S (2001). Dendritic glutamate autoreceptors modulate signal processing in rat mitral cells. *J Neurophysiol* **85**, 1275–1282.

Sassoe-Pognetto M & Ottersen OP (2000). Organization of ionotropic glutamate receptors at dendrodendritic synapses in the rat olfactory bulb. *J Neurosci* **20**, 2192–2201.

Schaefer AT, Angelo K, Spors H & Margrie TW (2006). Neuronal oscillations enhance stimulus discrimination by ensuring action potential precision. *PLoS Biol* **4**, e163.

Schaefer AT & Margrie TW (2007). Spatiotemporal representations in the olfactory system. *Trends Neurosci* **30**, 92–100.

Schoppa NE & Westbrook GL (2001). Glomerulus-specific synchronization of mitral cells in the olfactory bulb. *Neuron* **31**, 639–651.

Schoppa NE & Westbrook GL (2002). AMPA autoreceptors drive correlated spiking in olfactory bulb glomeruli. *Nat Neurosci* **5**, 1194–1202.

Sohl G, Maxeiner S & Willecke K (2005). Expression and functions of neuronal gap junctions. *Nat Rev Neurosci* **6**, 191–200.

Sullivan RM, Stackenwalt G, Nasr F, Lemon C & Wilson DA (2000). Association of an odor with activation of olfactory bulb noradrenergic β-receptors or locus coeruleus stimulation is sufficient to produce learned approach responses to that odor in neonatal rats. *Behav Neurosci* **114**, 957–962.

Suzuki N & Bekkers JM (2006). Neural coding by two classes of principal cells in the mouse piriform cortex. *J Neurosci* **26**, 11938–11947.

Uchida N & Mainen ZF (2003). Speed and accuracy of olfactory discrimination in the rat. *Nat Neurosci* **6**, 1224–1229.

Urban NN & Sakmann B (2002). Reciprocal intraglomerular excitation and intra- and interglomerular lateral inhibition between mouse olfactory bulb mitral cells. *J Physiol* **542**, 355–367.

Verhagen JV, Wesson DW, Netoff TI, White JA & Wachowiak M (2007). Sniffing controls an adaptive filter of sensory input to the olfactory bulb. *Nat Neurosci* **10**, 631–639.

Wilson DA, Fletcher ML & Sullivan RM (2004). Acetylcholine and olfactory perceptual learning. *Learn Mem* **11**, 28–34.

Wilson RI & Nicoll RA (2002). Endocannabinoid signaling in the brain. *Science* **296**, 678–682.

Zucker RS & Regehr WG (2002). Short-term synaptic plasticity. *Annu Rev Physiol* **64**, 355–405.

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