

Neuropharmacology of the Olfactory Bulb

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Abstract: The olfactory bulb is located at the start of a hierarchical chain of sensory processing mechanisms. The relative ease of its isolation allows the possibility that models of these mechanisms might be integrated to develop a detailed understanding of function. In this sensory processing chain odour molecules evoke signal transduction in the olfactory receptor neurons. These signals represent the diverse range of molecular binding affinities of the olfactory receptor proteins. The first level of processing of this sensory input is performed by the neurons of the olfactory bulb. The olfactory system needs to filter the vast amount of sensory input it receives to be able to select the subset of biological significance. The importance of the olfactory bulb in this filtering process is suggested by its wide range of modulatory mechanisms. These mechanisms include an array of centrifugal inputs from other regions of the brain as well as numerous intrinsic feedback circuits. Given the complexity of the olfactory bulb and the range of its modulatory activity, the process of isolation of its components produces some difficulties of interpretation. This is mainly due to the removal of some of the effects of interaction and the change in balance that results. We present a summary of the current understanding of the interacting modulatory elements that are found in the olfactory bulb and a detailed account of the properties of mitral/tufted cells, the projection neurons of the olfactory bulb. This is followed by a discussion of the intrinsic and extrinsic modulatory systems acting on these cells. A consideration of the integration of the effects of these modulatory systems allows an understanding of how the output of the mitral/tufted cells is controlled.

While significant progress has been made in the elucidation of the individual components as a result of advances in techniques over the last decade we suggest that there is a need for computational studies as a further aid to the understanding and interpretation of the weight of individual modulatory components in this dynamic interacting system.

Keywords: Olfaction, olfactory bulb, granule cell, mitral cell, mitral/tufted cell, glomerulus, olfactory receptor neuron, olfactory neuron, oscillations.

INTRODUCTION

The olfactory bulb has a tightly packed laminar structure (see Box 1) in which many different populations of neurons interact to carry out the integration and processing of the sensory input. The complex structure of the olfactory bulb has required that researchers use many inventive strategies to isolate the different interactions and interpret the role they serve. This work has revealed an abundance of sophisticated modulatory systems, which makes olfactory bulb neuropharmacology challenging to interpret and reflects the degree of analytical processing of the sensory input that occurs at this stage of the olfactory system.

Olfactory stimuli are almost always complex mixtures but are, in the main, perceived as a single odour object. How this is accomplished can be approached theoretically by building mathematical models to generate the observed output from a given input [1] or experimentally by examining the effects of simple mixtures on cellular activity [2]. Another approach is to understand how the individual circuits in the olfactory bulb function as signal processing units. Olfactory bulb neuropharmacology is the fundamental basis of such an understanding. In this review we highlight the complex relationships between competing excitatory and inhibitory modulatory mechanisms within the neural circuits of the olfactory bulb. Added to this complexity is the multi-channel nature of the input and the additional temporal dimension. An example of such complexity are oscillations which have been a consistent focus in olfactory bulb electrophysiology since the early work of Adrian in the 1940s and 50s. The idea of temporal coding in which the firing pattern of the oscillations carries odour information has become increasingly important [3, 4]. The electrophysiological and neurophysiological origins of these oscillations continue to be a strong theme in current research [5, 6].

THE FIRST SYNAPSE IN THE OLFACTORY SYSTEM

The first synapse of the olfactory system occurs at the glomerulus (see Fig. 1) where up to 1000 axons from olfactory receptor

neurons terminate. Glutamate is the main excitatory transmitter of the olfactory system and the mitral/tufted cell receptors that are stimulated by the release of glutamate from the olfactory receptor neuron axon terminals are a highly heterogeneous population [7]. External tufted cells can be distinguished from the mitral cells on functional grounds. A synchronising oscillation (~2Hz) of the juxtglomerular cells is attributed to the mitral cells [8]. This aside, in this paper mitral cells will mean those cells whose somata lie in the mitral cell layer and whose size is reasonably uniform. The convention in the literature is to refer to "mitral/tufted" cells because it is difficult to be certain that electrodes have been inserted in cells that are completely representative of the narrower definition of mitral cells. There is evidence in mitral/tufted cells for the presence of AMPA, kainate and NMDA receptors (see Fig. 1), as responses isolated at the olfactory receptor neuron-mitral/tufted cell synapse can be separated into a fast CNQX sensitive (AMPA/kainate) component and a slower AP5 sensitive (NMDA) component [9-11]. Surgical isolation of the glomerular layer does not abolish either component, confirming that currents in the glomeruli are responsible for these olfactory receptor neuron evoked responses as opposed to a polysynaptic response in the granule cells [12].

The excitation of mitral/tufted cells is phasic in nature, oscillating in the theta wave band. At least part of this is the effect of the rhythmic cycles of respiration and sniffing on olfactory receptor neuron stimulation driven by centrifugal input [13, 14]. However if odour exposure of the epithelium is artificial (i.e. not rhythmic), controlled phasic firing is reduced but not abolished [15]. The kinetics of this synapse therefore play a role in the period of the excitation, with the AMPA/kainate receptors depolarising the mitral cells sufficiently to overcome the Mg^{2+} block of the NMDA receptors. The NMDA receptors then depolarise the mitral/tufted cells further, once the AMPA/kainate receptors have inactivated. The period of excitation is thought to be extended further in a regenerative process in which glutamate is released by the mitral/tufted cells, which auto-excites glutamate receptors on the primary dendrite of the mitral/tufted cells [16, 17]. The period of excitation is terminated when the mitral/tufted cells are hyperpolarized by feedback inhibition from granule cells (see below).

The population of approximately 50-100 mitral/tufted cells that send their primary dendrites to the same glomerulus [18] have syn-

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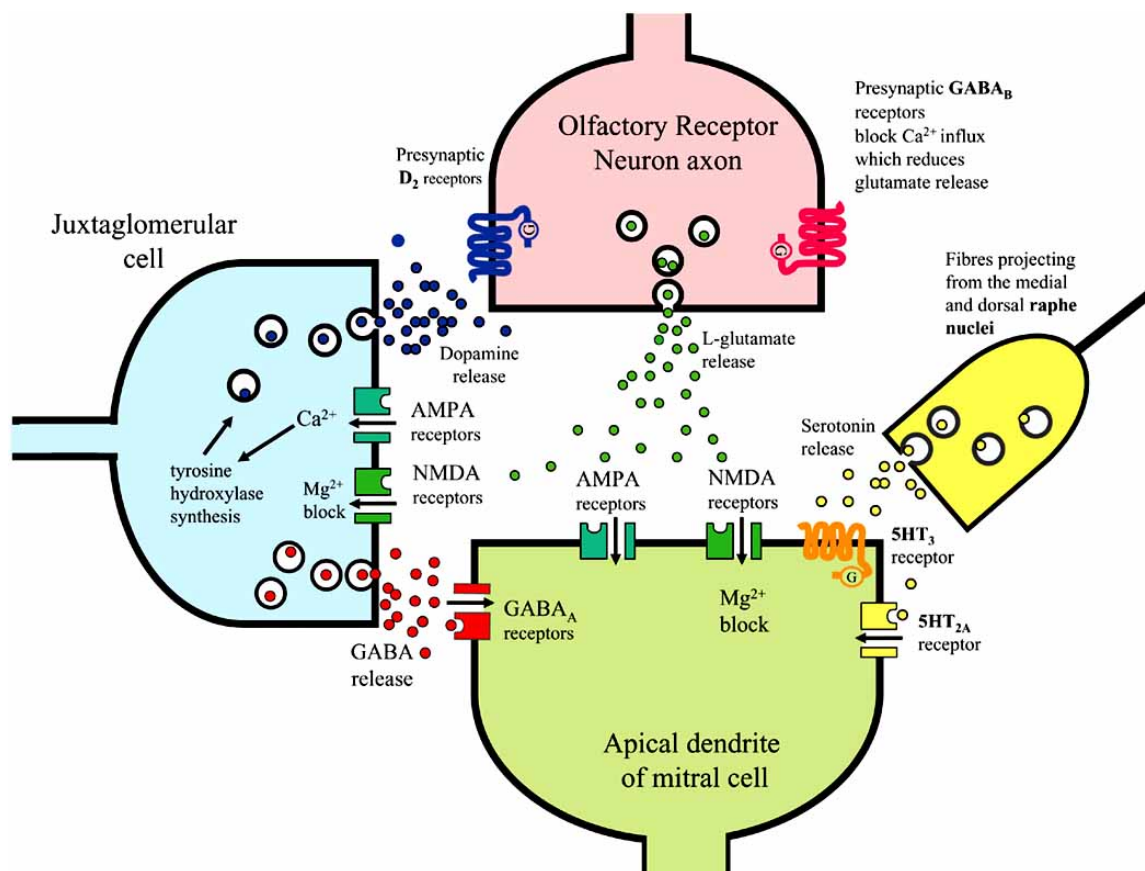


Fig. (1). Glomerular synapses showing the variety of receptors. The axons from the olfactory receptor neurons form the olfactory nerve which synapses on the primary apical dendrites of the mitral cells. L-glutamate is the primary excitatory transmitter at this synapse which binds to AMPA and NMDA receptors on the postsynaptic membrane. Juxtglomerular cells are inhibitory GABAergic/dopaminergic interneurons that mediate inhibition between glomeruli. Centrifugal fibres project from the Raphe nuclei to the glomeruli modulating the mitral cell activity *via* postsynaptic 5HT receptors (see text).

chronised response patterns [17]. This synchronisation is thought to be provided *via* gap junctions [19-21]. This would justify the complex mechanism slowing mitral/tufted cell oscillations, as slow oscillations facilitate the correlation of signals. The oscillations are slowed by the kinetics of the depolarization of the first synapse in the olfactory bulb. Gap junctions will synchronise a population of mitral cells with apical dendrites terminating in the same glomerulus. With slow oscillation the chance of two mitral cells being synchronized by chance firing is much reduced [19] and the whole process of synchronisation is further enhanced because populations of mitral cells need to work in unison to overcome NMDA block by Mg²⁺.

The Active Properties of Mitral/Tufted Cells

The mitral/tufted cells generate action potentials that initiate close to the soma in response to weak olfactory nerve input, but with stronger synaptic excitation the site of action potential initiation shifts to the distal dendrites [22, 23]. The ability to initiate spikes in the distal dendrites is likely to be due to the higher than normal sodium channel densities to be found in the mitral/tufted cell primary dendrites (90 pS/μm²) [24, 25]. The action potential back propagates into both the primary and secondary dendrites triggering calcium transients in all regions as P/Q- and to a lesser extent N-type calcium channels open [26]. The action potential attenuates along the secondary dendrites probably due to the hyperpolarizing effect of an A-type potassium current [27]. However no attenuation of the calcium transients is seen along the dendrites, extending to the distal tips [28].

There is evidence that further currents modulate mitral cell activity including:

- A sub-threshold oscillation of mitral/tufted cell membrane potential, thought to synchronise spike firing in populations of mitral cells and to be mediated by a non-inactivating sodium current I_{NaP} [29].
- Spike clustering in mitral/tufted cells which is thought to depend on the interplay between slowly inactivating I_D-like K⁺ channels and a sub-threshold TTX-sensitive Na⁺ current [30].
- Local control of dendritic excitability which is mediated by small conductance calcium activated potassium (SK) channels [31].

As well as the theta oscillations (~2Hz) mentioned above, originating in the glomeruli and synchronised for mitral cells projecting to the same glomerulus [17], spontaneous subthreshold oscillations in membrane potential have been observed in mitral cells at resting membrane potential [29]. The frequency was found to be 10 Hz near their spike threshold and rose to 20 Hz at the spike threshold. Further depolarization of the cell progressively increased the frequency of subthreshold oscillations up to 40 Hz at -59 mV [29]. In addition, task related gamma oscillations in the olfactory bulb have been observed [32-36] in which the mitral cells are thought to participate. Further discussion of oscillations is beyond the scope of this review and the reader is referred to the work cited in this section.

INTRINSIC MODULATION IN THE GLOMERULUS

Each glomerulus is a functional unit receiving convergent input from olfactory receptor neurons expressing a particular receptor protein [37]. In a recent review Chen and Shepherd [38] have sug-

gested that the key functional operation of the glomerulus is to act as a signal-to-noise enhancing device in the processing of sensory input.

Modulation of Olfactory Nerve Glomerular Input

The output from each functional unit is provided by a population of 50-100 mitral/tufted cells and this output is modulated by a population of 1500-2000 juxtglomerular cells (Fig. 1 and Box 1). Based on their response to depolarizing pulses, individual juxtglomerular cells can be divided into two physiological classes: bursting and standard firing [39] of which the former (mainly periglomerular external tufted cells) oscillate spontaneously. Based on their mode of firing and placement in the bulb circuit, these bursting cells are well situated to drive synchronous oscillations in the olfactory bulb and it has been suggested that juxtglomerular cells are responsible for spontaneous glomerular field layer potentials [40] which may represent one level, or sub-system, of processing in the olfactory bulb network [40]. Juxtglomerular cells include periglomerular, external tufted and short axon cells. It is a heterogeneous population that is slowly being divided into subpopulations that differ chemically, morphologically and physiologically. Much more work needs to be done in characterising these cells and the function they serve (the current extent of the knowledge of juxtglomerular cells is reviewed in Kosaka and Kosaka [41]). Approximately 50% of juxtglomerular cells that have been partially characterised so far can be divided into the following populations, some of which overlap:

GABAergic Periglomerular Cells

Populations of GABAergic periglomerular cells interact with both olfactory nerve axon terminals and mitral/tufted cell dendrites in the glomerulus [42] (see Fig. 1). The GABAergic population of periglomerular cells in the rat constitutes about 20% of the cells in the glomerular layer. GABA released from the periglomerular cells, combined with the rapid action of the GABA_A receptors and a postsynaptic position (see Fig. 1) on the mitral cells [43], allows sufficient hyperpolarisation of the mitral/tufted cell apical dendrites to cancel the effect of remaining L-glutamate in the synaptic cleft. A presynaptic location (see Fig. 1) of the slower but longer duration action of the GABA_B receptor on the olfactory nerve terminals [43, 44] allows the blocking of further L-glutamate release until the GABA_B effects wear off. Only about 20% of periglomerular cells receive monosynaptic olfactory nerve input, and it may be this population that mediates presynaptic inhibition of the olfactory nerve [45]. This presynaptic inhibition appears to extend the dynamic range of the post-synaptic bulbar neurons as well as exerting some feedback control over the very strong excitatory drive from the incoming axons, particularly as increasing odour concentrations drive these thousands of olfactory receptor cells to higher firing frequencies [38].

There is also likely to be interneuron-to-interneuron inhibition as functional GABA_A receptor channels are found in periglomerular cells [46]. Stimulation of periglomerular cells in the rat olfactory bulb results in self-inhibition as the GABA activates GABA_A receptors on the same neuron as well as spilling over to neighbouring periglomerular cells [47]. The periglomerular cells represent the first level of inhibition in the olfactory system and this GABA-mediated self-inhibition will result in mitral/tufted cell excitation during intense olfactory stimulation [47].

Dopaminergic Cells

The glomerular layer (see Box 1) of the olfactory bulb contains one of the largest populations of dopaminergic neurons in the brain and dopamine in the olfactory bulb is found exclusively in the juxtglomerular neurons [48]. A subset of GABAergic periglomerular

cells contain dopamine and tyrosine hydroxylase, a critical enzyme in dopamine production [41, 49, 50]. The dopamine released by this population of periglomerular cells is thought to reduce olfactory nerve output, because dopamine application reduced the olfactory nerve evoked response in mitral/tufted cells mediated by a presynaptic D2 receptor [9]. Both D2 and D1 receptors are expressed in the olfactory bulb but D2 receptors are the predominant sub-type and are expressed in the terminals of olfactory nerve axons (see Fig. 1) and in presynaptic elements of the glomerular neuropil, including mitral/tufted cell dendrites and the dendrites of a subset of GABAergic/dopaminergic periglomerular cells [51]. D1 receptors are only sparsely expressed in the rat olfactory bulb (glomerular layer, external plexiform, mitral and granule cell layers), stimulate cAMP and are excitatory, whereas D2 receptors reduce cAMP and are inhibitory.

GABAergic/dopaminergic periglomerular cells and dopaminergic periglomerular cells are auto-rhythmic in the theta wave band [46, 52]. Tyrosine hydroxylase expression in these cells is reduced following both peripheral deafferentation of the olfactory bulb [53] or unilateral naris (nostril) closure [54]. In tissue culture experiments it has been shown that both odour stimulation of olfactory receptor neurons and glutamate release by the olfactory receptor neurons provokes tyrosine hydroxylase induction; a process mediated by NMDA receptors followed by the activation of L-type Ca²⁺ channels and an increase in intracellular calcium ion concentration [55]. In this way dopaminergic periglomerular cells allow activity-dependent modulation of inhibition over a longer term than the GABAergic periglomerular cells. The purpose of this mechanism appears to be linked with discrimination of odours as it was found that individual mitral/tufted cells were three times more likely to respond to both citral and peppermint after odour deprivation or injection of the dopamine antagonist spiperone [56]. Mice which lacked a functional D2 receptor, or in which the D2 receptor had been knocked out, exhibited impaired odour discrimination and reversal learning but were normal for odour habituation, sensitivity and odour recognition memory [57, 58]. Knocking out the dopamine transporter (DAT^{-/-}) results in disturbances in extracellular dopamine and apparently down-regulated D2 receptor expression and gives rise to a similar phenotype as the D2 receptor knock out [58].

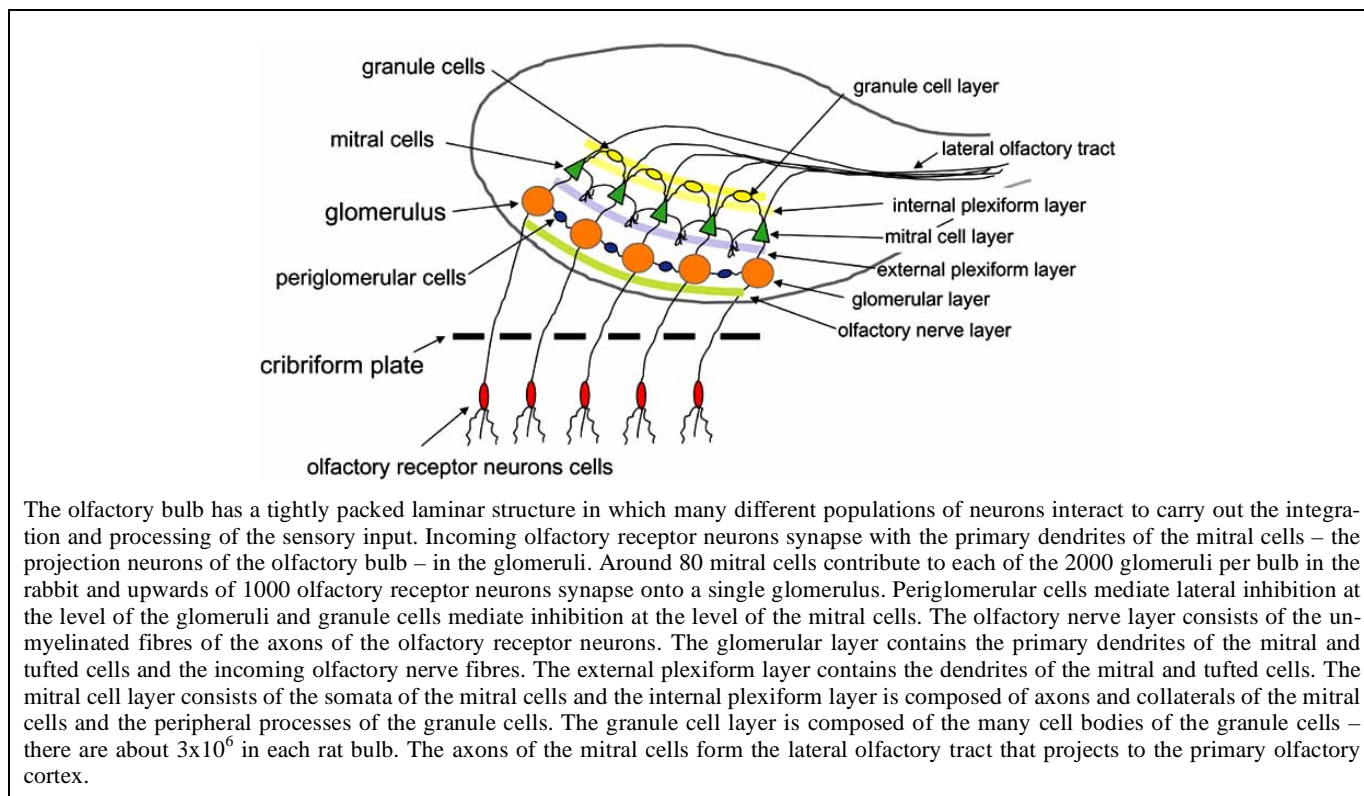
Dopaminergic cells also send dendrites into non-olfactory nerve regions where they contact dendrites from mitral/tufted cells and centrifugal axons [52].

NO Containing Cells

Nitric oxide is another transmitter that is slowly revealing a specialised modulatory mechanism amongst periglomerular cells. Nitric oxide being gaseous in nature diffuses much more readily than other transmitters. It is produced by neuronal nitric oxide synthase, which has been found in a distinct population of periglomerular cells [59] thought to be GABAergic [60]. It has been suggested that the target of the periglomerular nitric oxide transmitter is another population of periglomerular cells that express the β_1 subunit of soluble guanylyl cyclase, and also mostly appear to express calbindin D28K [61]. The activation of the soluble guanylyl cyclase by the nitric oxide will result in the production of the intracellular messenger cGMP. The interplay of nitric oxide and cGMP triggers molecular mechanisms, including adaptation processes, which enable the olfactory neuroepithelium to cope with strong stimuli [62].

External Tufted Cells

The external tufted cells receive direct input from the olfactory nerve axon terminals and in turn provide intraglomerular monosynaptic excitatory input to many short axon and periglomerular interneurons that do not receive direct olfactory nerve input [45]. External tufted cells express slowly inactivating sodium channels



Box 1. The Layers of the Olfactory Bulb.

The olfactory bulb has a tightly packed laminar structure in which many different populations of neurons interact to carry out the integration and processing of the sensory input. Incoming olfactory receptor neurons synapse with the primary dendrites of the mitral cells – the projection neurons of the olfactory bulb – in the glomeruli. Around 80 mitral cells contribute to each of the 2000 glomeruli per bulb in the rabbit and upwards of 1000 olfactory receptor neurons synapse onto a single glomerulus. Periglomerular cells mediate lateral inhibition at the level of the glomeruli and granule cells mediate inhibition at the level of the mitral cells. The olfactory nerve layer consists of the unmyelinated fibres of the axons of the olfactory receptor neurons. The glomerular layer contains the primary dendrites of the mitral and tufted cells and the incoming olfactory nerve fibres. The external plexiform layer contains the dendrites of the mitral and tufted cells. The mitral cell layer consists of the somata of the mitral cells and the internal plexiform layer is composed of axons and collaterals of the mitral cells and the peripheral processes of the granule cells. The granule cell layer is composed of the many cell bodies of the granule cells – there are about 3×10^6 in each rat bulb. The axons of the mitral cells form the lateral olfactory tract that projects to the primary olfactory cortex.

(I_{NAP}), which generate rhythmic spike bursts that are readily entrained by olfactory nerve input [8]. In this way the external tufted cells act as a glomerular synchronising mechanism [63]. Bursting external tufted cells oscillate spontaneously and their position and electrical activity make them well situated to drive synchronous oscillations in the olfactory bulb [39]. The oscillatory activity of external tufted cells is finely tuned by endogenous GABA and glutamate [63].

Because of their bursting properties external tufted cells may amplify sensory input onto the glomerular network, possibly enabling synchronization of the network, including mitral cells, to sensory input, which is in turn synchronised to sniffing [8].

Short Axon Cells

The short axon cells send interglomerular axons over long distances to form excitatory synapses with inhibitory periglomerular neurons 20-30 glomeruli away [64]. Interglomerular excitation of these periglomerular cells potently inhibits mitral cells and forms an on-centre, off-surround circuit. This interglomerular centre-surround inhibitory network, along with the well-established mitral–granule–mitral inhibitory circuit, forms a serial, two-stage inhibitory circuit that could enhance spatiotemporal responses to odours [64].

INTRINSIC MODULATORY CIRCUITS IN THE EXTERNAL PLEXIFORM LAYER

The lateral dendrites of mitral/tufted cells make dendrodendritic reciprocal synapses with granule cells (Fig. 2). This local synaptic circuit forms the basis for reciprocal dendrodendritic inhibition mediated by ionotropic GABA_A receptors in mitral cells [65]. This interaction is responsible for generating the synchronised γ -oscillatory activity (30-70Hz) in the olfactory bulb which is thought to be important in odour detection and discrimination.

The dendrodendritic synapses are host to a heterogeneous population of AMPA/kainate and NMDA receptors [66, 67]. In situ hybridization experiments have suggested that mitral/tufted cells express kainate receptors of the type GluR5 and KA2, whereas interneurons (periglomerular and granule cells) express mostly GluR6 and KA2 [68]. Granule cells also express high levels of metabotropic glutamate receptors. On the basis of inhibitor studies, Heinbockel *et al.* [69] suggest these are mGluR5, the activation of which participates in feedforward and/or feedback inhibition at mitral/tufted cell to granule cell dendrodendritic synapses, possibly to modulate lateral inhibition and contrast in the olfactory bulb. Release of L-glutamate by the lateral dendrites of mitral/tufted cells stimulates the granule cells in a process mediated by this battery of receptors. The resulting long lasting two-component depolarisation of the granule cells provokes GABA release [70]. Under this feedback inhibition repetitive stimulation will result in a lower frequency of depolarisations as the inhibition blocks further episodes of depolarisation until a recovery period has elapsed [71].

The AMPA/kainate receptors have fast kinetics so can be identified as the initial component of the granule cell EPSC, whereas the long lasting slower component is due to the activation of NMDA receptors [66]. The AMPA/kainate kinetics are too fast to overcome the hyperpolarisation caused by the transient A-type potassium channel current (I_A) so these receptors produce insufficient depolarisation to provoke spiking or GABA release [72]. Rather, the main function of these non-NMDA receptor channels is to depolarise the granule cells in order to facilitate NMDA activation by overcoming the Mg^{2+} block of the NMDA receptors [73]. As previously noted, mitral/tufted cells act in synchronised populations and so the convergence of many synchronised mitral/tufted cells on to individual granule cells further facilitates the lifting of the Mg^{2+} block [74]. The kinetics of the NMDA receptors are sufficiently slow that once I_A has inactivated, the resultant depolarisation is sufficiently deep to not only promote spiking [72], but also to activate N and P/Q type high voltage Ca^{2+} channels [26]. So I_A me-

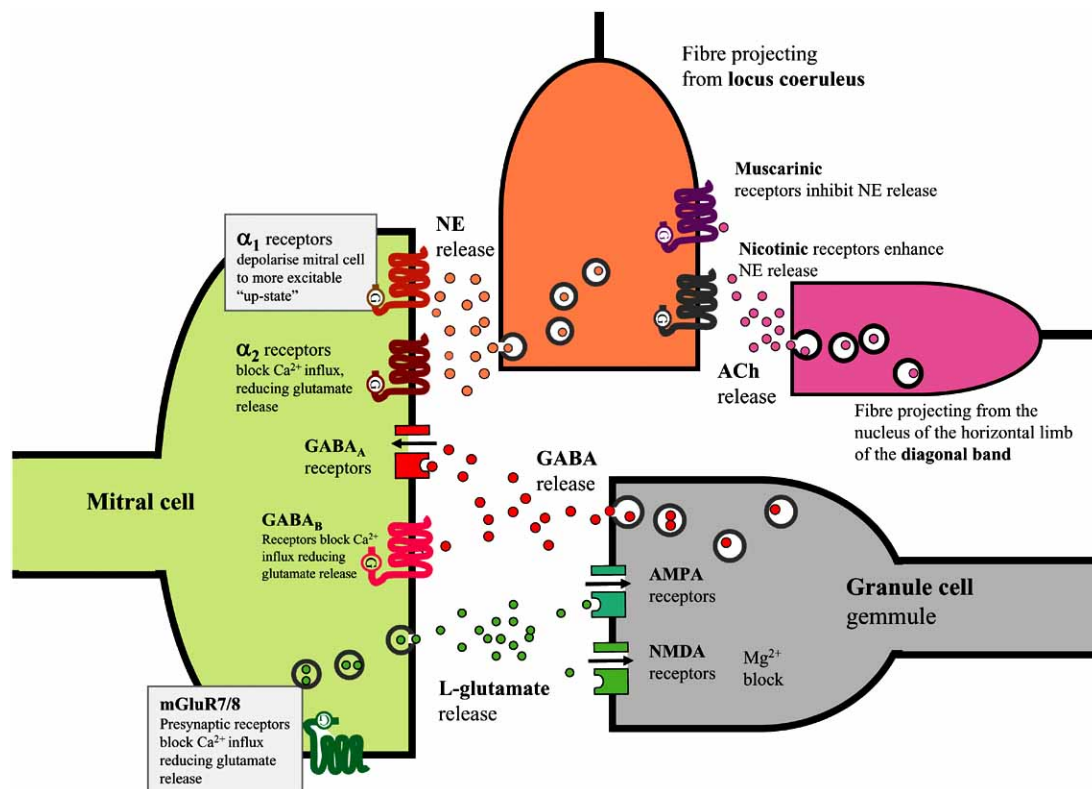


Fig. (2). Synapses in the external plexiform layer of the olfactory bulb showing the variety of receptors and neurotransmitters. Granule cells mediate feedback and lateral inhibition between mitral cells with which they form reciprocal dendrodendritic synapses. Adrenergic and cholinergic efferent fibres project from the diagonal band and the locus coeruleus respectively (see text for further explanation).

diates a controlling mechanism that allows the granule cells to generate GABAergic inhibition of mitral cells [72]. The granule cell is well adapted to promote this retarding shift in kinetics, with high densities of I_A channels in the distal dendrites, and low densities of somatic sodium channels [72, 75]. The rapid kinetics of AMPA receptors, together with the inactivation of I_A , ensures that granule cells have short spike-response times and that they are able to synchronize rapidly, resulting in phase-locked GABA release on to mitral cells [76]. The elevation of local intracellular Ca^{2+} concentration in granule cells mediated by activation of the N and P/Q channels provokes exocytosis of GABA [26].

Recently two functionally distinct excitatory synapses have been found on granule cells [77]; distal synapses which are the dendrodendritic inputs from mitral cells with slow kinetics, exhibiting paired-pulse depression, and proximal axonal inputs with fast kinetics, exhibiting facilitation [30]. These proximal synapses originate from two sources, local axon collaterals from mitral cells and centrifugal feedback projections from cortical regions. This input, originating from the piriform cortex, synapses onto granule cells and can gate dendrodendritic inhibition onto mitral cells [30]. The implications of this are that the degree of lateral inhibition in the olfactory bulb after sensory stimulation may be dynamically modulated by activity in the piriform cortex [30].

There is evidence for heterogeneity in the inhibition imposed on the lateral dendrites since, as well as the GABA mediated inhibition, glycine and taurine have both been found to inhibit mitral/tufted cells [78, 79]. Also, GABA_B receptors have been found on granule cells which may act as autoreceptors [65].

EXTRINSIC MODULATION OF THE GLOMERULUS AND MITRAL CELLS

The projection of serotonergic fibres from the median and dorsal Raphe nuclei passes along the ventromedial surface of the ante-

rior olfactory nucleus and then enters the olfactory bulb from its exterior surface, together with the olfactory nerves [80]. The fibres terminate densely in the glomeruli, and less densely in the infra-glomerular layers [80]. Serotonergic fibres in the glomeruli are thicker, contain more numerous and larger varicosities, and are more intensely stained than most infraglomerular fibres [80]. Deafferentation of olfactory bulb serotonergic fibres causes olfactory disturbance in the short term and shrinkage of the glomerulus in the longer term [81].

The development of odour memory to conditioned odour training is associated with the phosphorylation of cAMP response element binding (CREB) protein in the rat neonatal olfactory bulb [82]. This type of odour conditioning has also been linked to 5-HT (serotonin) receptors [83], and β -adrenoceptors [84], as well as extrinsic noradrenergic modulation (see below). 5-HT receptors of the sub-classes 5-HT_{2A} [85] and 5-HT₃ [86] have been found using immunocytochemistry and selective antagonist binding respectively. 5-HT (serotonin) receptors are co-localised with β -adrenoceptors on mitral cells [86]. Beta-adrenergic stimulation increases cAMP in mitral cells – an effect that requires 5-HT-induced mobilization of Ca^{2+} . A model of odour induced learning has been suggested in which the convergence of these effects with odour stimulation recruits CREB phosphorylation and induces memory-associated changes in the olfactory bulb [87].

EXTRINSIC MODULATION OF CIRCUITS IN THE EXTERNAL PLEXIFORM LAYER AND GRANULE CELL LAYER

Extrinsic Glutamatergic Modulation

Centrifugal fibres from the olfactory cortex [88] and anterior olfactory nucleus may make excitatory synapses with granule cells. The granule cells would then impose inhibitory output on mi-

tral/tufted cells. The anterior olfactory nucleus projections pass *via* the anterior commissure to the contralateral bulb [89]. Another possibility is that these centrifugal fibres have a direct modulatory effect by releasing zinc directly on sites postsynaptic to glutamate release [90].

Extrinsic Noradrenergic Modulation

Noradrenergic fibres project from the locus coeruleus to the granule cell layer *via* the lateral olfactory tract [91] (see Fig. 2). An early study demonstrated that noradrenaline reduces the inhibition exerted by the granule cells on the mitral cells [92]. Evidence has been gathered for the expression of α_1 , α_2 , and β -adrenergic receptors in relation to the lateral dendrite synapses. Alpha-1 receptors have been found on mitral/tufted cells that cause a G-protein mediated inhibition of leak potassium current. The resultant depolarisation, although modest, was sufficient to lift the mitral cell to an activated state that would make the cell more responsive to olfactory nerve stimuli [93]. Alpha-2 receptors have been demonstrated on the mitral/tufted cell lateral dendrites that block high threshold Ca^{2+} currents *via* a G-protein mechanism. This causes presynaptic blocking of glutamate release and the net result is loss of feedback inhibition from granule cells [94, 95].

A β -receptor mediated mitral cell inward current was identified using the agonist isoproterenol. However this current could be abolished by pharmacological isolation with TTX, AP5, CNQX, and gabazine, indicating that it was most likely a circuit effect [93]. The β -receptors were found to mediate a sequence that consisted first of a depression of the feedback inhibition imposed on mitral/tufted cells followed by a potentiation of this inhibition [96]. The β -receptors have also been implicated in olfactory learning as odour conditioning in young rats that is normally reinforced by tactile stimulation was reinforced by the β -receptor agonist isoproterenol [84]. This mechanism also appears to involve disinhibition of the mitral/tufted cells from the GABAergic inhibition of the granule cells [97].

It has been shown that association of odour with a surge of noradrenalin from the locus coeruleus to the olfactory bulb is sufficient to produce a subsequent relative preference for that odour in rat pups. Pairing an odour with either direct activation of noradrenergic β -receptors within the olfactory bulb or with putative direct pharmacological stimulation of the locus coeruleus produces a subsequent relative odour preference in a dose-dependent manner [98].

Extrinsic Cholinergic Modulation

Cholinergic fibres project to the olfactory bulb from the horizontal limb of the diagonal band [99, 100]. A population of juxtglomerular cells and a population of cells in the granule cell layer (Fig. 2) are amongst the recipients of the cholinergic projection [101]. The receptors receiving the cholinergic input were found to have distinct areas of expression, with nicotinic receptors found mainly in the glomerulus producing short-lived excitation of a population of juxtglomerular cells, and a longer lived excitation of mitral cells [102]. Muscarinic receptors inhibit granule cell firing rate while increasing their activity dependent GABA release [102] an effect mediated by pirenzepine-sensitive M1 receptors.

Rats with selective lesions of cholinergic neurons that project to the olfactory bulb and cortex discriminate less well between aliphatic aldehydes with similar carbon chain lengths than do rats that received sham lesions [103].

The noradrenergic projection appears to be under the control of the cholinergic projection, as stimulation of muscarinic receptors on the noradrenergic fibres inhibits noradrenaline release and stimulation of nicotinic receptors promotes noradrenaline release [104]. The net effect of acetylcholine administration appears to favour the nicotinic response as the result is noradrenaline release [104].

Extrinsic Modulation by Orexin

Fibres originating from the lateral and posterior hypothalamus of the rat containing the peptides orexin A and B (molecules that regulate food intake and arousal state) were distributed in the glomerular layer, mitral cell layer and granule cell layer layers (see Box 1) of the olfactory bulb [105]. Type 1 orexin receptor expression has been found in the rat olfactory bulb from 10 days to adult in periglomerular, mitral/tufted cells and granule cells [105]. Depolarisation and hyperpolarisation of mitral cells has been observed along with changes in mitral cell firing rates and responsiveness to food and non-food odours with the application of orexin A [105, 106]. Apfelbaum *et al.* [106] suggested that this change in firing activity could underlie a larger scale regulation of the olfactory system, allowing the threshold for odour detection to be modified depending on the nutritional status of the animal and the responses to food and to non-food odours near threshold to become more salient. They also pointed out that orexin may be involved in the well-documented changes of responsiveness induced by the nutritional state.

These inhibitory local circuits within the olfactory bulb serve a number of functions in addition to the more conventional lateral inhibition for "centre-surround" sharpening of the response to a given stimulus. For example, they give rise to the generation of bursts in firing frequency and oscillatory activity. They are responsible for the temporal variation in these firing patterns and oscillations and the synchronization and desynchronization of oscillations in mitral/tufted cells. They interconnect mitral/tufted cells that can be some distance apart and underlie the phenomenon of "declustering" [4] that occurs following extended exposure to an odour.

CIRCADIAN MODULATION OF THE OLFACTORY BULB

A circadian oscillation in the responsiveness of the olfactory bulb has been observed in rats [107] and mice [108]. In mice the peak responsiveness of mitral cells to odour stimulation occurred during the subjective night and persisted under constant dark conditions as well as following lesions in the suprachiasmatic nucleus, suggesting that the olfactory bulb contains an autonomous circadian pacemaker [108]. In humans, odor-evoked event-related potentials peak during the day, being largest at around 16:00 and smallest at 04:00 [109].

ENDOCRINE MODULATION OF THE OLFACTORY BULB

Oxytocin

Maternal behaviour in rats appears to be promoted by an odour imprinting mechanism that involves oxytocin mediated changes in the olfactory bulb [110]. The effect of oxytocin appears to be mediated by a presynaptic mechanism affecting GABA release from granule cells [111]. Microiontophoretic administration of oxytocin inhibited mitral cells and excited granule cells by an augmentation of glutamatergic transmission. This may enhance the self-inhibition of active mitral/tufted cells or their lateral inhibition on less active neighbours through the reciprocal dendrodendritic synapses, thereby mediating several aspects of maternal and social behaviour which could rely on the gating of olfactory input (such as odour imprinting and nipple search behaviour) [112].

Insulin and Brain Derived Neurotrophic Factor

Insulin suppresses the voltage dependent outward current in cultured olfactory bulb mitral and granule cells, an effect that is mimicked by Src tyrosine kinase [113]. This modulation was thought to be mediated by tyrosine kinase phosphorylation of potassium Kv1.3 channels [113]. It is possible that insulin in the brain could also be used as a satiety factor. After a meal, blood insulin

levels would rise in response to increased serum levels of glucose but, in the olfactory bulb, insulin levels are low after a meal. As mentioned above, the threshold for odour detection is dependent upon nutritional status; thus mitral cell firing rates, influenced by insulin levels, could represent a potential mechanism for this effect [114].

Chronic exposure to brain derived neurotrophic factor (BDNF) increased the magnitude of Kv1.3 current mediated by a Trk receptor kinase [115]. Mitral cells in Kv1.3 null mice failed to show normal modulation of currents by these tyrosine kinase mediated mechanisms, but also there were other side effects including an increased ability to discriminate between odorants [116].

KNOCKOUT MODELS OF THE OLFACTORY BULB

Another method for examining the function of specific mechanisms in the olfactory bulb is to delete the gene of interest. This is a relatively new field that is providing some interesting insights. The importance of the granule cells in odour discrimination was clearly demonstrated in a neural cell adhesion molecule-deficient mouse model. In these mice the rostral migratory stream is interrupted resulting in a 40% size reduction of the olfactory bulb. This reduction was restricted to the granule cell layer, a layer that contains the GABAergic interneurons. The mice with this deficit had reduced odour discrimination but their detection threshold for odours and their short-term odour memory were unimpaired, demonstrating that the granule cells are crucial only for odour discrimination but not for general olfactory functions [117]. In the normal olfactory bulb the mitral cells within a particular glomerulus exhibit synchronised activity; knocking out the gap junction gene Cx36 completely eliminated this synchronised activity [118]. Correlated spiking between mitral cells is a mechanism for maintaining the fidelity of the glomerular activation map induced by a particular odorant.

Receptor knockouts have been used to investigate the role of the D2 dopamine receptor [58] (see above under "Dopaminergic cells"), the GABA_A receptor [119], the insulin receptor [120], group 1 metabotropic glutamate receptors [121, 122] and the rho 1 subunit of the GABA-C receptor [123].

Disruption of the GABA_A receptor resulted in mitral/tufted cells with increased mIPSCs and significant increases in theta and gamma oscillations. In odour discrimination tasks, the knockout mice became better than their wild type counterparts in distinguishing closely related monomolecular alcohols. However, the null mice were initially better and became worse than control mice at distinguishing closely related mixtures of alcohols. Disruption of GABA_A receptor mediated synaptic inhibition of GABAergic interneurons and the augmentation of IPSCs in the mitral/tufted cells resulted in increased network oscillations in the olfactory bulb with complex effects on olfactory discrimination related to the size or power of oscillating networks [119]. Knockout of the GABA_C rho 1 subunit resulted in increased smell sensitivity [123] and knockout of the metabotropic glutamate receptor, mGluR5, demonstrated that activation of mGluR5 participates in feedforward and/or feedback inhibition at mitral cell to granule cell dendrodendritic synapses, possibly to modulate lateral inhibition and contrast in the olfactory bulb [122].

Transgenic technology, in which genes are deleted (knockouts), either completely or conditionally, or are knocked in, offers a powerful method for investigating the contribution of specific mechanisms to the function of the entire olfactory bulb. The consequences of such genetic manipulations can then be observed at the cellular, organ, system or behavioural level. However, to interpret the results of such interventions requires the development of accurate models of olfactory bulb function. It is therefore the future partnership of genetic and computer modelling technologies that will begin to provide the integrated solution to the problem of how we smell.

CONCLUDING REMARKS

The immediate impression that one gains when considering the complexities of the neuropharmacology of the olfactory bulb is that each system employs multiple backups. Does this complexity tell us that the olfactory system is so important to the survival of mammals that an element of redundancy has been built in as a fail-safe? Alternatively, the complexities of the modulation may be part of a highly sophisticated multidimensional processing system that we have just not found the key to interpreting.

The answers to these questions are to some extent thwarted by the available methods for research in neuropharmacology where the emphasis is on characterising the components of a system by a process of isolation. In the process interaction between systems is lost. Even exploring the effects of gene-targeted deletion, a technique that aims to side-step the isolation issue, can result in the establishment of a new equilibrium rather than an interpretable system failure. How then is it possible to examine the way the many modulating components interact? A possible answer to this question and something that will inevitably become a greater part of future research in neuropharmacology is realistic neuronal modelling. Computational models of olfactory bulb signal processing have contributed to our understanding of several aspects of olfactory function; for example, filtering and contrast enhancement, mechanisms underlying oscillation and spike synchronization and odour discrimination and associative memory function [124]. Some of the references quoted show how far this process has already come, but there are so many interactions occurring between the different modulatory systems that have been isolated, that this should be a highly fertile field for future research. If such modelling is to start to achieve anything worthwhile, the other disciplines will have to collaborate more with modellers and become much more quantitative in their approach. The more data on parameters that is available to modellers, the more meaningful will be the interpretations or predictions that they are able to produce.

Considerable progress has been achieved in the isolation of different components of the complex mechanisms of the olfactory bulb. With the right balanced approach to further research in this area in-roads can be made into interpreting how the components interact and what functions are served by these interactions.

ABBREVIATIONS

AMPA	=	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP5	=	2-amino-5-phosphonopentanoate (NMDA receptor antagonist)
cAMP	=	Cyclic adenosine monophosphate
cGMP	=	Cyclic guanosine monophosphate
CNQX	=	6-cyano-7-nitroquinoxaline-2,3-dione (competitive AMPA/kainate receptor antagonist)
CREB	=	cAMP response element-binding
EPSC	=	Excitatory post-synaptic potential
GABA	=	γ -aminobutyric acid
5HT	=	5-hydroxytryptamine (serotonin)
IPSC	=	Inhibitory post-synaptic current
mGluR	=	Metabotropic glutamate receptor
NMDA	=	N-methyl-D-aspartic acid
NO	=	Nitric oxide
TTX	=	Tetrodotoxin

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Received: November 19, 2007

Revised: April 17, 2008

Accepted: April 23, 2008