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Vomeronasal/Accessory Olfactory System and Pheromonal Recognition

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Abstract

Pregnancy block in mice requires exposure of recently mated females to urinary pheromones of a strange male, and when working with inbred strains this invariably requires urine from an outbred line. The pheromones which induce oestrus and early puberty in mice have been identified as the brevicomins and dihydrothiazoles. Since the same vomeronasal, neural and neuroendocrine pathways are also activated in pregnancy block, these compounds are likely candidates for pregnancy blocking pheromones. However, these relatively simple chemicals lack the capacity to code for differing mouse strains. Since large quantities of the polymorphic major urinary proteins from the lipocalin family found in urine serve as transporters for the dihydrothiazoles and brevicomins, and differ across strains, then these proteins must participate in pheromone recognition in the context of pregnancy block.

The reproductive biology of rodents is strongly influenced by chemical cues, in the context of both behaviour (signalling pheromones) and reproductive endocrinology (primer pheromones). An important effect of primer pheromones, observed in the female, is the control of oestrus. This occurs in a number of species by invoking the onset of puberty (Vandenbergh, 1969) and the onset of oestrus after a period of anoestrus (Whitten, 1966). A special case of oestrus control which occurs in the mouse and a few other rodent species is the olfactory block to pregnancy. This was first described by Bruce (1959), who found that newly mated female mice returned to oestrus if they were exposed to strange males within 72 h of the initial mating. The fact that pregnancy block cannot occur after implantation suggests that the effect of male primer pheromones is on the pre-implantation hormonal status. In both the block to pregnancy and the induction of oestrus, the primary endocrine change is a fall in serum prolactin (Reynolds and Keverne, 1979; Ryan and Schwartz, 1980; Keverne, 1982; Marchlewska-Koj, 1983). Evidence that prolactin is the hormone mainly responsible for pregnancy block comes from experiments which show that restricted exposure of female mice to primer pheromones coincident with prolactin surges following mating blocks pregnancy by changing hypothalamic dopamine release (Rosser *et al.*, 1989), while electrical stimulation of the mouse accessory olfactory bulb, also coincident with prolactin surges, does the same (Li *et al.*, 1994).

In parallel to the common neuroendocrine mechanisms for oestrous induction, all of the pheromone effects described above involve the vomeronasal/accessory olfactory system. Lesions to the vomeronasal organ or to the accessory olfactory bulb prevent female odours from inducing anoestrus and male odours from inducing oestrus, accelerating puberty onset and blocking pregnancy (Keverne, 1983). Although I use the term 'odours', it is extremely unlikely that these pheromones are volatile since they elute chemically as peptides, and hence females must make contact with male urine (Vandenbergh *et al.*, 1975). Contact with urine is also essential to stimulate the receptors in the vomeronasal organ, and it is thought that the pumping action of the VNO during licking and nuzzling facilitates access of chemical stimulants (Meredith and O'Connell, 1979). Hence, these pheromones exert their effects via a common neural pathway, namely the accessory olfactory system, with their receptors in the vomeronasal organ. The tuberoinfundibular dopaminergic neurons (TIDA) in the arcuate nucleus of the hypothalamus represent the final common pathway. This neural pathway been identified by electrophysiolgical stimulation of the AOB and recording from neurons identified in the arcuate nucleus by antidromic stimulation of the median eminence (Li *et al.*, 1989). These neurons have been identified as dopaminergic using the neurotoxin 60HDA to selectively lesion TIDA arcuate neurons (Li *et al.*, 1990).

At both the neural and neuroendocrine levels, the pheromonal mechanism for inducing pregnancy block has much in common with pheromonal mechanisms for promoting early puberty and inducing oestrus in grouped females. Since these occur in response to urinary pheromones from

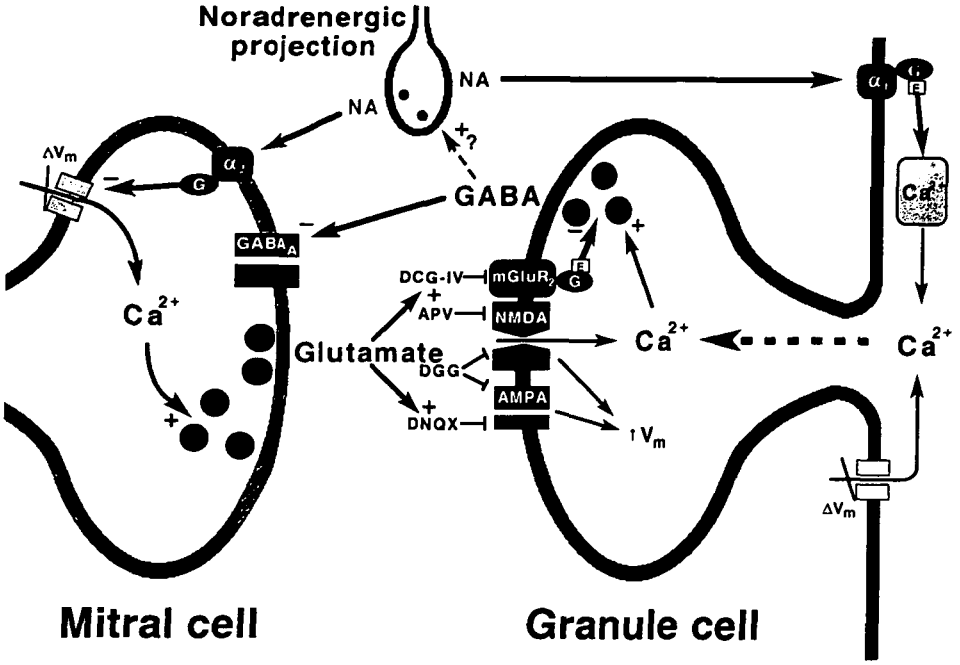

Figure 1 Schematic presentation of mitral–granule cell interactions in the female following mating. Glutamate acts on the granule cell via NMDA, AMPA and metabotropic receptors. Both NMDA and AMPA receptors require blocking in order to prevent memory formation. Electrophysiological recordings show NMDA receptor activation to be involved in the slow component of the granule cell response, while EPSPs are completely blocked when both receptors are antagonized. Activation of the metabotropic receptor ($MGluR_2$) will induce memory formation, but not if AMPA receptors are blocked. Noradrenaline (NA) is thought to have both presynaptic (mitral) and postsynaptic (granule cell) effects. Presynaptically there is a reduction in spontaneous mitral cell inhibitory post-synaptic potentials induced by GABA. Postsynaptically NA causes a rapid rise in Ca^{2+} in granule cells. Hence, under the influence of NA during birth or mating, the mitral cell firing frequency increases (disinhibition from granule cell) while changes in intracellular Ca^{2+} produce long-term intracellular modifications of the granule cell, the outcome of which is an altered firing frequency of mitral cells. APV, NMDA channel blocker; DNQX, AMPA channel blocker; DCGIV, $MGluR_2$ agonist.

any male, the question arises as to why pregnancy block only occurs with strange male pheromones. Mechanisms appear to exist which bring about recognition and subsequent gating of the pheromonal signal from the familiar male. Several features which characterize the neural basis of this olfactory recognition memory have now been elucidated. It is known that memory formation occurs in a critical period after mating (Keverne and de la Riva, 1982); this is a function of the vomeronasal accessory system (Bellringer *et al.*, 1980; Lloyd-Thomas and Keverne, 1982) and is dependent on noradrenergic innervation of the AOB (Rosser and Keverne, 1985). A series of studies has shown that the relatively primitive structure of the AOB has the capacity for synaptic changes of importance for the recognition memory and subsequent gating of biologically significant odours (Brennan *et al.*, 1990). Moreover, electrophysiological studies have identified the external plexiform layer of the AOB to be particularly significant in this context (Kaba and Keverne, 1992). Orthodromic stimulation of the VNO leads to highly reproducible potentials in the AOB, while current source-density analysis of these field potentials has revealed the laminar and temporal distribution of synaptic currents. In combination with pharmacological manipulations, these studies have revealed two major synaptic inward currents. The initial change of events is in the glomerular layer and underlies excitatory post-synaptic potentials (EPSPs) evoked in the glomerular arbors of mitral cells. Subsequent changes in the external plexiform layer underlies EPSPs in the granule cells via the AMPA glutamate receptors, but not NMDA receptors. A series of pharmacological studies involving in-vivo bilateral infusions of drugs into the AOB during the critical period for memory formation of male pheromones have revealed the synaptic events that are important for recognition memory (summarized in Figure 1). In-vivo microdialysis of transmitter release confirmed these findings (Brennan *et al.*, 1995) and supports the view that the critical changes occur at the mitral-granule cell dendrodendritic synapse (Figure 1).

Although the synaptic events which undergo changes at mating in the presence of pheromone stimulation are critical to recognition memory, it is the pattern of activity across the population of mitral and granule cells that determines which male pheromones are recognized. We have examined the expression of the immediate early genes *c-fos*, *c-jun* and *egr-1* in the mitral and granule cells of the accessory olfactory bulb immediately after mating (Brennan *et al.*, 1992). Increases were observed in the number of mitral and granule

cells transiently expressing *c-fos* and the number of mitral and granule cells expressing *egr-1* during the period of memory formation. No changes were seen in the expression of *c-jun* during this period. The increase in the number of cells expressing *c-fos* and *egr-1* required the association of mating and pheromonal exposure, conditions also required for memory formation. A tenfold increase in mitral cells expressing *egr-1* and granule cells expressing *c-fos* was observed when bicucculin was infused into the AOB in the presence of pheromones, which is consistent with the effects this drug has on the formation of non-specific memories.

From these studies it appeared that the code for individual recognition is carried by the pattern of activity across the population of mitral cells, which in turn is determined by the specificity of ligand–receptor interaction in the vomeronasal receptors. However, of the ligands identified with pheromonal activity in mice [brevicomins, dihydrothiazoles, farnesones and heptanones (Navotny *et al.*, 1990)], none have the capacity to code for individual recognition. Nevertheless, mice secrete in their urine a specific group of proteins that are involved in binding volatile molecules. These proteins belong to the lipocalyn family (major urinary proteins; MUPS), have a large gene family with many allelic variants (Clarke *et al.*, 1985) and the total urinary pool of MUPs is highly polymorphic (Clissold *et al.*, 1982). These urinary proteins (5–20 mg produced daily) have been crystallized and have been shown by X-ray crystallography to bind pheromones (Bocskei *et al.*, 1992). When MUPs are purified from urine they are associated with a number of ligands (brevicomins, thiazoles); indeed, almost all of these compounds are protein bound with very little free in the aqueous phase (Bacchini *et al.*, 1992).

Since no single pheromone ligand can possibly code for an individual's identity but the MUP transporter proteins do have this polymorphic capacity, the MUP proteins probably code for individual recognition in the context of pregnancy block. One possibility is that the MUP protein presents the ligand to the VNO receptor, in a manner similar to the T cell in the immune system, and takes an active part in the transduction process. However, a common feature of the seven transmembrane receptors that bind proteins is an extensive N-terminal. Mouse VNO receptors do not appear to have a long N-terminal, although few have been completely characterized to date. In which case, an alternative mechanism for pregnancy block could be its induction by a cocktail of the pheromone ligands, which bind to the MUP proteins in a variety of ways, thereby differentially exposing selected parts of the pheromone for receptor binding. However, with the VNO receptors cloned and sequenced, together with the known pheromone ligands and transporter, it should be possible to test these propositions in expression vectors *in vitro*.

In addition to the non-volatile nature of VNO ligands through their coupling to MUP proteins, the receptors of the VNO also differ from the main olfactory receptors. The gene family that codes for VNO receptors belong to the seven-transmembrane family but is unrelated to that of the main olfactory odour receptors (Dulac and Axel, 1995). Moreover, the patterning of odour receptors in the main olfactory epithelium has a bilaterally organized topography, with a single receptor type projecting to a single spatially distinct glomerulus (Vassar *et al.*, 1995). The VNO receptor neurons in the mouse appear not to be organized with bilateral symmetry, and functional studies, using immediate early genes as markers in the AOB, would suggest that the response to an inbred line of mouse pheromones differs across individuals. Hence the rigorous hard-wired topography of receptor neuron projections to the main bulb appears to have no obvious parallel in VNO to AOB projection.

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