# Olfactory Recognition: A Simple Memory System

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**Mice have an olfactory (pheromone) recognition memory located at the first relay in the sensory system. It is acquired with one-trial learning, contingent upon norepinephrine activation at mating, and lasts for several weeks. The mechanism involves Hebbian (association-dependent) changes in synaptic efficacy at dendrodendritic synapses in the accessory olfactory bulb. As a result of this memory, males made familiar by mating are recognized by the females, thereby mitigating pregnancy block. Such a memory function is biologically important to the female, as it is required to sustain pregnancy in the presence of her stud male's odors.**

MAMMALIAN PHEROMONES INFLUENCE A WIDE VARIETY of behaviors and physiological states (*1*). One of the most compelling effects regulated by pheromones is the olfactory block to pregnancy. Pregnancy block occurs before implantation if recently mated female mice are exposed to the urine of strange males (*2*). In the laboratory this is readily demonstrated with urine from a strain of mice different from that of the male that mated (*3*). Various hypotheses have been put forward to explain the adaptive significance of the pregnancy block phenomenon, ranging from male competition and promotion of exogamy to a protection against mate desertion or infanticidal males (*4*). Although appealing, none of these hypotheses take into account the mechanisms upon which evolutionary forces have acted.

At both the neural and endocrine level, the pheromonal mechanism for inducing pregnancy block has much in common with pheromonal mechanisms for promoting early puberty and inducing estrus in grouped females (*5*). The latter are of adaptive value to the female, but since they are brought about by pheromones from any male, strange or familiar, this raises the question as to why pregnancy block only occurs with a strange male. Presumably some mechanism exists to bring about recognition and subsequent gating of the pheromonal signal from the familiar male. Certainly, it is these familiar pheromones, and not those from males of a different strain, that are likely to be in the female's environment during the vulnerable period leading up to implantation (*6*). Hence, by recognition of familiar pheromones, pregnancy loss is normally avoided. It is this recognition memory that is discussed here. We consider the temporal characteristics of memory formation and duration, its neural and synaptic bases, and what these may tell us about learning and memory in general.

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## Temporal Features

Mating normally results in 90 to 100% of female mice becoming pregnant, and this level is sustained regardless of whether or not the female remains with her stud male or his odor-soiled bedding. If she is removed 6 hours after mating and returned at any future time, pregnancy is sustained, but pregnancy fails if she is placed with a strange male or his pheromones. If the female is removed immediately after mating and returned to the original mate some 6 hours later, implantation fails to occur, resulting in only 10 to 20% of pregnancies being maintained (*7*). These findings thus imply that familiarity is contingent upon mating, but requires a prolonged exposure of 4 to 6 hours to the male's pheromones in order for subsequent recognition to occur, the critical period being immediately after mating.

Other studies have considered the duration of this recognition memory (*8*). This was investigated by first blocking pregnancy with a strange male and then allowing this male to mate after varying time intervals before reexposure to the original mate's pheromones to test for recognition. The interval between the first and second matings was increased until the pheromones from the original mate blocked pregnancy. It could then be reasonably concluded that their memory trace had faded. It was found that pheromones from the original mate were unable to block pregnancy when they were introduced to the female, after a second mating, at intervals of 10, 20, and 30 days from the original mating. Between 80 and 90% of females still recognized the stud male's pheromones on reintroduction (Fig. 1). Pregnancy block was no greater than in the group exposed to the familiar male 24 hours after mating, suggesting that the olfactory memory was still present. However, when the second exposure to the original mate's pheromones occurred at an interval of 50 days, 70% of females experienced pregnancy block. This percentage of pregnancy block is similar to that which occurs upon exposure to a strange male and hence the original, familiar male is now responded to as strange, implying that the duration of the memory trace is 30 to 50 days.

## Neural Systems

Recognition, particularly of something as complex as odors of different strains of mice, might be thought to be represented by the cortical neuronal systems to which the main olfactory bulb (MOB) projects. We were unable to show with our experiments that recognition, in the context of pregnancy block, was a function of the main olfactory system. Female discrimination of normal male urine from castrate male urine (which does not contain these pheromones) was severely impaired by selective lesions to the receptors of the main olfactory system (*9*). But the ability of strange male urine to block pregnancy, in contrast to familiar male urine, was unchanged by these lesions. In other words, recognition memory, in the context of pregnancy block, proceeded as normal without the main olfactory receptors. Selective destruction of the vomeronasal organ had the converse effect; it prevented pregnancy block but was without influence on urine discrimination (*9*). Thus, in the context of pregnancy block, the process of recognition that occurs after mating is a function of the vomeronasal system and its central projections.

The central projections of the vomeronasal system are distinct from those of the main olfactory system (*10*) (Fig. 2). Whereas the latter projects principally to cortical structures, the vomeronasal projections are subcortical to the neuroendocrine hypothalamus via the amygdala (*11*). The amygdala also interacts with the hippocampus, and hippocampal lesions produce rapid forgetting of olfactory information (*12*). Nevertheless, lesions to the hippocampus were without effect on either the ability of strange males to block pregnancy or the ability of the female to recognize the familiar mate and thereby prevent pregnancy block (*13*). These findings support the view that the accessory olfactory system and its projections to the hypothalamus are necessary and sufficient, not only to produce the neuroendocrine changes necessary for pregnancy block, but also to sustain the process of recognition that occurs for the familiar mate.

The question arises as to which projection sites of the accessory olfactory system are required for the storage of the recognition memory to the mate's pheromones. To address this question, we infused the anesthetic lignocaine locally after mating at the first two relays of the accessory olfactory system (*14*). Such a procedure, when directed at the accessory olfactory bulb (AOB), prevented recognition and resulted in the mate's pheromones blocking his own pregnancy. However, when lignocaine was infused into the medial amygdala immediately after mating, there was no block to memory formation, and only strange males were able to disrupt the females' pregnancies. Since lignocaine infusions to the amygdala disabled transmission, but did not prevent memory formation, it is clear that the recognition memory must be occurring at an earlier point in the pathway than the amygdala. Taken together with all of the necessary control procedures, which examined the nonspecific effects of infusions and the spontaneous occurrence of abortions, these experiments point to the AOB itself as an important site, not only for the recognition process, but also for the memory trace that prevents the familiar mate from blocking his own pregnancy. Therefore this structure became the focus for our attention in addressing the following questions: What signals to the AOB start the critical sensitive period? What plastic changes in the AOB enable the recognition memory to be formed?

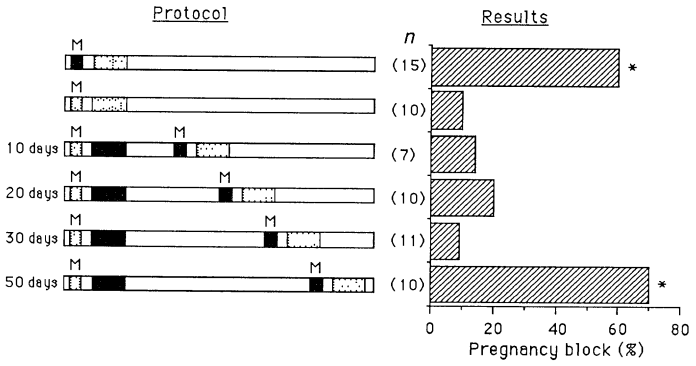



**Fig. 1.** Duration of recognition memory: M, mating; dotted block, exposure to BALB/c male; filled block, exposure to F1 (C57 × CBA) male. (See text for details; $*P < 0.01$, information statistic.)

## Signaling of the Critical Period

The brain's noradrenergic projections form a neural subsystem that identifies situations of survival value by providing the instructions for the storage of relevant sensory information (*15*). The granule cell and external plexiform layers of both the MOB and AOB receive a rich norepinephrine (NE) projection from the locus ceruleus (*16*). A number of models have been developed to examine the significance of these NE terminals in the olfactory bulb for olfactory learning. In the rabbit, NE has been shown to be of critical importance in shifts of electroencephalographic activity in the MOB during associative odor learning (*17*). NE has also been implicated in the associative learning of odors coupled with tactile stimulation in rat pups (*18*).

Our own studies have shown that formation of the olfactory memory to pheromones is dependent on centrifugal NE projections to the AOB (*19*). Selective lesions to this system before mating prevent the formation of the recognition memory and result in the stud male's blocking of his own pregnancy (*7*). Moreover, mating induces a significant increase in NE turnover in the MOB and AOB, but not in the cortex of the mouse. This increased activity at NE terminals lasts for at least 4 hours after mating, correlating with the exposure time to pheromones that is required to form the memory. Moreover, at 48 hours after mating, when reexposure to the stud male fails to block pregnancy, there was no enhanced NE turnover. This would imply that increased activity in NE terminals is not necessary for memory recall. This was confirmed by lesion studies made after mating, which did not affect the maintenance of pregnancy or recognition of the familiar male. Further studies have revealed that blockade of α- but not β-adrenergic receptors also prevents memory formation, if the NE blocker phentolamine is infused immediately after mating (*20*).

## Synaptic Mechanisms

Later studies have investigated the effects of drug infusions into the AOB on the recognition memory formed by female mice to male pheromones (*20*). This model has the advantage that the experiments are performed on freely behaving animals, and, because of the established structure of the AOB, the effects of the drugs infused locally can be attributed to actions at specific synapses. Failure to form a memory leads to an effect not revealed previously, and the test for memory formation involves an unambiguous physiological end point: the animals are either pregnant or not pregnant.

The synaptic circuitry of the AOB is comparatively simple and is very similar to that of the MOB (*21*) (Fig. 3). Mitral cells receive afferents from the vomeronasal nerve and project to the medial amygdala, forming the excitatory pathway to the hypothalamus for pheromonal signals received by the vomeronasal organ receptors. The mitral cells form reciprocal dendrodendritic synapses with granule cells, the main class of interneuron in the AOB. Granule cell

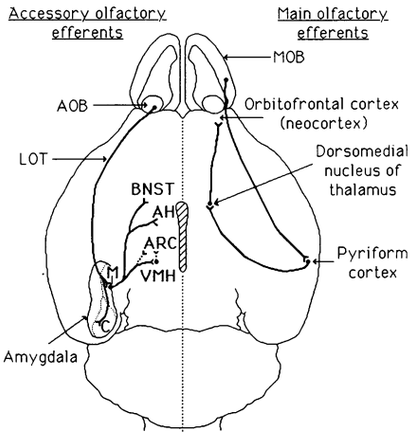
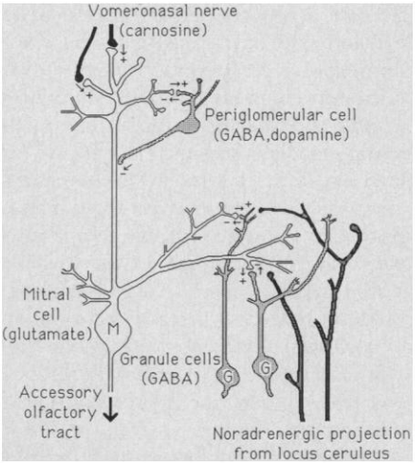



**Fig. 2.** A comparison between the central projections of the main olfactory bulb and the accessory olfactory bulb: AOB, accessory olfactory bulb; AH, anterior hypothalamus; ARC, arcuate nucleus; BNST, bed nucleus of the stria terminals; C and M, cortical and medial nuclei of the amygdala; LOT, lateral olfactory tract; MOB, main olfactory bulb; VMH, ventromedial hypothalamus.

**Fig. 3.** Synaptic relations and major neurotransmitters of the AOB.




synapses are depolarized by an excitatory amino acid input from the mitral cells, and in turn provide a feedback inhibition to the mitral cells via γ-aminobutyric acid (GABA) release. This interaction between mitral and granule cells at the reciprocal synapse regulates mitral cell activity by feedback inhibition.

Intracellular recordings, in the turtle olfactory bulb, have clearly demonstrated that NE reduces the inhibition exerted by the granule cells on the mitral cells (*22*), and this has been interpreted as enhancing the signal-to-noise ratio. If NE reduces the feedback inhibition of granule to mitral cell, then sustained excitation of mitral cells will occur. This will produce a prolonged activation at the dendrodendritic synapses of a subset of granule cells over the 4-hour period of NE release and pheromone activation. This sustained excitation of granule cells might be mimicked experimentally by blocking the feedback inhibition of mitral cells with a GABA antagonist (*20*). If this blockade of feedback inhibition is sustained for a prolonged period when the mitral cells are active, it should be possible to create an olfactory memory without mating. An experiment was designed to address this issue by infusing bicucculline bilaterally into the AOB of estrus females at 0 and 1.5 hours during a 6-hour exposure to BALB/c male bedding, without mating (Fig. 4). At the next estrus, females were mated with either a CBA or a BALB/c male and then were reexposed to male pheromones of either the BALB/c or CBA strains to test for recognition (*8*). Blockade of feedback inhibition to mitral cells, during pheromonal exposure, did indeed create an olfactory memory (Fig. 4). However, this memory lacks the specificity of the memory formed at mating and appears to generalize to the pheromones of at least one other strain (CBA). This absence of specificity in recognition probably arises as a result of the bicuculline infusion blocking feedback inhibition to the majority of mitral cells, causing widespread excitation of the associated granule cells. In contrast, mating-released NE "imprints" only that subset of granule cell synapses that is associated with the active population of mitral cells, the specificity probably being increased by local enhancement of NE release (*23*).

A model of synaptic plasticity that is widely studied is long-term potentation (LTP) in the hippocampus (*24*). Although few studies have directly related this to any specific process of memory formation, it has been used as a model to study mechanisms of synaptic plasticity that might form the basis of memory processes (*25*). In the CA1 region of the hippocampus, the establishment of LTP at pyramidal cell synapses is dependent on stimulation of the *N*-methyl-D-aspartate (NMDA) type of excitatory amino acid receptor (*26*). However, the maintenance phase of LTP and its expression do not require activation of NMDA receptors and seem to be mediated by non-NMDA excitatory amino acid receptors (*27*). In another region of the hippocampus, the synapse of mossy fiber to CA3 pyramidal cell, the establishment of LTP seems not to be dependent on NMDA receptors and is instead mediated by non-NMDA receptors (*28*).

An excitatory amino acid neurotransmitter, thought to be glutamate, aspartate (*21*), or the dipeptide *N*-acetylaspartylglutamate (*29*), mediates the transmission from mitral to granule cells. Therefore, we have investigated the effects of selective (NMDA) and nonselective excitatory amino acid antagonists on the recognition memory (*30*). Local infusions of the nonselective antagonist γ-D-glutamylglycine (DGG) into the AOB, during the critical period, prevented formation of a recognition memory to the stud male. However, infusions of the selective, competitive NMDA antagonist D-2-amino-5-phosphonovaleric acid failed to prevent memory formation. This suggests that antagonism of the NMDA receptor alone is not sufficient to interfere with the formation of the memory to the stud male. This conclusion is further supported by the ineffectiveness in preventing memory formation of systemic injections of MK-801, a highly selective and noncompetitive NMDA antagonist. Therefore, the process of synaptic plasticity, by which a female mouse forms a memory to the pheromones of the stud male, may be dependent on the stimulation of non-NMDA excitatory amino acid receptors. In this respect, the model has characteristics similar to LTP at the synapse of mossy fiber to CA3 pyramidal cell in the hippocampus. The similarity is further supported by the dependence of LTP in this region of the hippocampus on NE (*31*).

## Conclusions

Although a number of studies have dealt with synaptic plasticity in a variety of learning contexts, few have considered situations that

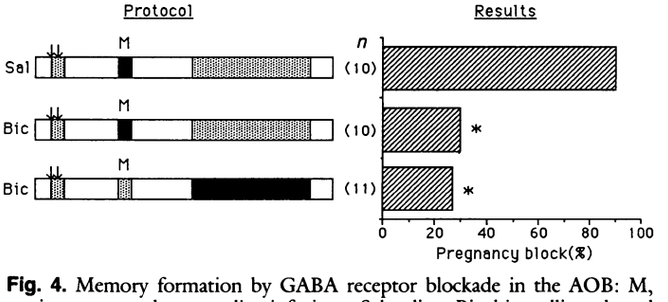



**Fig. 4.** Memory formation by GABA receptor blockade in the AOB: M, mating; arrows, drug or saline infusions; Sal, saline; Bic, bicuculline; dotted block, exposure to BALB/c male; filled block, exposure to CBA male. (See text for details; $*P < 0.001$, information statistic.)

are biologically relevant to species survival. The studies discussed here implicate the mitral-granule cell dendrodendritic synapse in the formation of a recognition memory. The association of NE and pheromonally induced activity, during the critical period following mating, is postulated to cause a lasting change in the efficacy of the mitral-granule cell reciprocal synapse. After this period, when the level of NE has fallen back to premating levels, this population of mitral cells would be subject to an increased inhibitory component from their associated granule cells. On subsequent stimulation, the patterning of mitral cell activity matching that encountered after mating will thus be selectively gated (failure of the stud male to block pregnancy), whereas patterns of activity that are substantially different will not. Hence, pheromones from strange males acting on a population of mitral cells without increased feedback inhibition will still promote the neuroendocrine mechanisms that result in pregnancy failure.

Recognition of an odor usually infers a memory for that odor. It has long been supposed that memories consist of "traces" or "engrams" left in the brain by previous experiences. Work over the past decade has demonstrated that humans have different forms of memory (*32*). The distinction between these forms of memory is obviously of significance because it raises the possibility that different brain structures may be involved in different test situations. The findings reported here show that the relatively primitive trilaminar structure of the AOB has the capacity for synaptic changes of importance for the recognition and subsequent gating of biologically significant odors. Not only has evolution been conservative in its neural mechanisms for memory [the combination of noradrenergic, GABA-ergic, and excitatory amino acid transmitters frequently feature in mechanisms of memory (*33*)], but in this case the changes occur at what is the most economic location, namely, the first neural relay in the sensory system.

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