NOTE *Ethology*

Activation of $GABA_A$ Receptors in the Accessory Olfactory Bulb Does Not Prevent the Formation of an Olfactory Memory in Mice

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ABSTRACT. When female mice are mated, they form a memory to the pheromonal signal of their male partner. The neural mechanisms underlying this memory involve changes at the reciprocal dendrodendritic synapses between glutamatergic mitral cells and γ-aminobutyric acid (GABA)-ergic granule cells in the accessory olfactory bulb (AOB). Blockade of $GABA_A$ receptors in the AOB leads to the formation of an olfactory memory. In an attempt to disrupt memory formation at mating, we used local infusions of the $GABA_A$ receptor agonist muscimol into the AOB during the critical period for memory formation. Muscimol across a wide range of doses (1–1000 pmol) did not prevent memory formation. The resistance of this memory to $GABA_A$ receptor activation may reflect the complexity of synaptic microcircuits in the AOB.

KEY WORDS: accessory olfactory bulb, $GABA_A$ receptor, olfactory memory.

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The reproductive physiology of mammals is strongly influenced by pheromones, with some of the most dramatic effects elicited in mice. Urinary male pheromones activate the vomeronasal system, by way of the vomeronasal receptors, and promote mating by inducing estrus in female mice [12]. However, if this pheromonal effect is induced within three days after mating, implantation of the embryos is prevented. This is referred to as the olfactory block to pregnancy and often occurs if a recently mated female is exposed to the pheromones of an unfamiliar male. To prevent the male partner from blocking pregnancy of his mate, the female forms a memory for the pheromones of her male partner. Several features which characterize the neural basis of this olfactory recognition memory have now been elucidated [1, 2, 8]. Memory formation depends on vaginocervical stimulation at mating [13], but requires a prolonged exposure of 4–6 hr to male pheromones immediately after mating [18]. The formation, but not recall, of the memory depends on mating-induced enhancement of α-adrenergic transmission in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system [7, 13, 18]. This memory lasts for at least 30 days following mating unless pregnancy ensues [11]. The neural changes underlying the long-lasting memory occur in the AOB [11].

Microcircuits in the AOB include the prominent reciprocal dendrodendritic synapse between mitral and granule cells. Glutamate released from mitral cell dendrites activates the dendrites of granule cells, which in turn mediate γ-aminobutyric acid (GABA)-ergic dendrodendritic inhibition back onto mitral cell dendrites [6]. This feedback inhibition at the reciprocal synapses regulates mitral cell activity [7, 15]. The formation of the pheromonal memory involves changes at the reciprocal synapses [3, 16]. The GABA-mediated feedback inhibition through the granule-to-mitral synapse is very powerful and is the main means for mediating control of output from the AOB, activating neuroendocrine mechanisms via the hypothalamus [7, 10, 15]. Blockade of $GABA_A$ receptors by local infusions of bicuculline, a $GABA_A$ receptor antagonist, into the AOB produces an olfactory memory without the occurrence of mating [11], suggesting that the relief of mitral cells from granule cell-mediated feedback inhibition is important for memory formation. We have therefore examined whether memory formation is disrupted by local infusions of muscimol, a $GABA_A$ receptor agonist, into the AOB during the critical period for memory formation.

The mice used in this study were adult males and virgin females of the Balb/c strain (Japan SLC, Hamamatsu, Japan) and CBA males (Charles River Japan, Atsugi, Japan). The animals were housed singly at 23°C with a reversed 12:12 hr light cycle (lights on 18.00, lights off 6.00). Fifty-three female mice were used for the study. Procedures conducted in the dark phase were under red light. Food and water were available *ad libitum*.

The estrous cycles of the females were monitored daily by taking vaginal smears. Mating was carried out naturally by placing an intact male into the home cage of a single estrous female. The majority of matings were completed 15–40 min later. To demonstrate pregnancy block, females were killed by cervical dislocation six days after mating and uterine horns were examined for implantation sites. The absence of implantation sites confirmed a positive pregnancy block.

Female Balb/c mice had permanently indwelling 25-gauge stainless steel cannulas stereotaxically implanted under Avertin (tribromoethanol) anesthesia at least 5 days prior to mating. In order to avoid trauma to the AOB, these

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cannulas were implanted just anterior to the AOB (co-ordinates relative to the intersection of the superficial sinuses and the surface of the olfactory bulb: longitudinal +0.5 mm, lateral ± 0.8 mm, vertical –0.3 mm).

For the purpose of investigating the local action of the $GABA_A$ receptor agonist muscimol (Sigma, St. Louis, MO, U.S.A.) in the AOB on the olfactory block to pregnancy, females received bilateral infusions of the drug at 0 and 1.5 hr after mating. In one group, infusions were made at 0, 1 and 2 hr after mating. The drug was infused over 5 min in a volume of 0.5 μl through 33-gauge stainless steel cannulas connected to manually operated 10-μl microsyringes. The infusion cannulas were left in place for one additional minute after infusion. Muscimol was dissolved in saline.

Cannulated females were allowed to mate with a Balb/c male. The females were left in the presence of the stud male's pheromones for 6 hr following mating before removal to a clean cage. On the afternoon of the day following mating, either the familiar (stud Balb/c) or strange (CBA) male was introduced to the female's cage and left with her for 48 hr. Four days later the females were killed and examined for implantation sites. Effectiveness of re-exposure (test exposure) to the familiar stud male in blocking pregnancy was used to evaluate whether memory formation had been prevented. When a low level of pregnancy block was obtained upon re-exposure to the familiar stud male, a comparison was made with the effectiveness of test exposure to strange male pheromones in blocking pregnancy. Data were analyzed with the Fisher exact probability test.

The results are summarized in Fig. 1. Two infusions of the $GABA_A$ receptor agonist muscimol across a range of doses (1–100 pmol) during the critical period for memory formation resulted in a low level of pregnancy block to the stud male (groups 2–4). For all three groups the level of pregnancy block was not significantly different from that for the saline-infused control (group 1) re-exposed to the stud male. The low level of pregnancy block could not be attributed to a disruptive effect of muscimol on the AOB structure because in females given 100 pmol muscimol a significant pregnancy block was induced by the strange male ($P<0.05$; group 5). Even the highest dose and frequency were still without effect on memory formation (group 6), despite the occurrence of drug-induced seizures. These results indicate that memory formation was not prevented by local infusions of muscimol into the AOB at any of the doses and frequencies used.

We had expected that infusions of the $GABA_A$ receptor agonist muscimol into the AOB during the critical period for memory formation would interfere with the formation of the pheromonal memory, so the resistance of this memory to activation of $GABA_A$ receptors was surprising. In relation to this, muscimol challenges to the AOB using the technique of *in vivo* microdialysis have been shown to increase the levels of noradrenaline without affecting the levels of excitatory neurotransmitters [3]. An increase in noradrenaline in response to muscimol challenges has also been reported in the sheep main olfactory bulb [14] and is probably due to the action of the $GABA_A$ receptor agonist on the noradrenergic presynaptic terminals [4]. Therefore, such local control of noradrenaline release by activation of $GABA_A$ receptors is probably important for enhancing the release of noradrenaline in the vicinity of the AOB mitral cells responding to male pheromones and thereby leading to the formation of the pheromone-specific memory as previously suggested [5]. This hypothesis is supported by the finding that bicuculline-induced memory lacks the pheromone specificity of the memory formed at mating and appears to generalize to

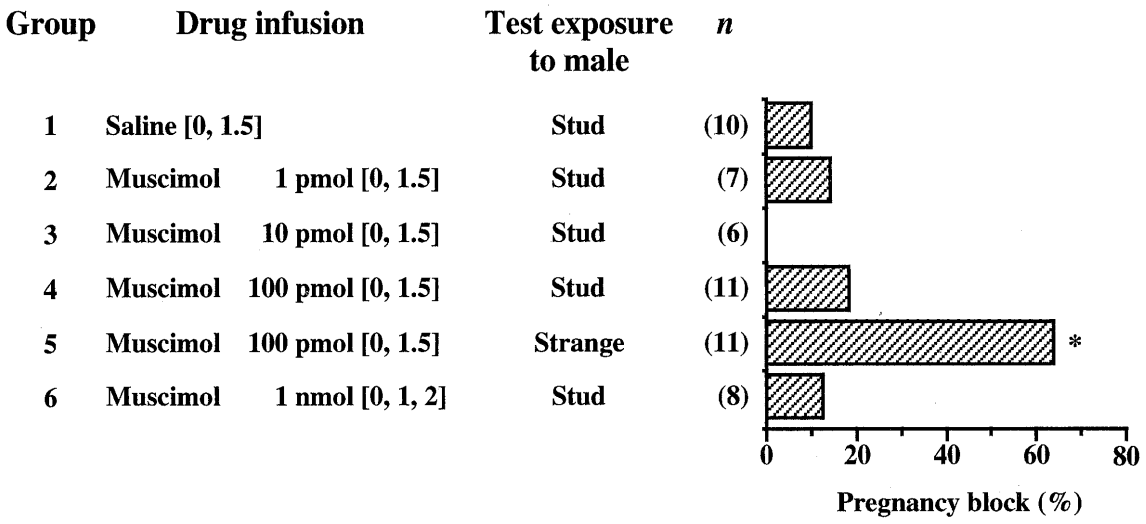

Fig. 1. Effects of local infusions of muscimol into the AOB on memory formation in the context of pregnancy block. Numbers in brackets refer to time (in hr) of drug infusions relative to mating. Numbers of animals tested are given in parentheses. * $P<0.05$ compared with the saline-infused group (Fisher exact probability test).

the pheromones of at least one other strain [11].

Pharmacological studies of pheromonal learning in the AOB have suggested that disrupting mating-induced memory formation is more difficult than creating an olfactory memory in the absence of mating [7, 9, 17]. This might reflect that multiple short-term exposures to male pheromones during the critical period after mating would be sufficient to produce a pheromonal memory. Accordingly, we cannot rule out the possibility that multiple short-term activation of mitral cells between muscimol infusions could have induced memory formation by circumventing the action of the $GABA_A$ receptor agonist.

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態にわずかな違いが見られるものの，両種の先体の形成における特徴は非常によく似たものであった．ジャワオオコウモリではステップ7以降で先体が徐々に伸長，扁平化し，最終的にスコップ状の形になるのに対し，コキクガシラコウモリではステップ8以降に先体が伸長，扁平化してわずかに退縮し，精子放出直前には先体が細長いへらのような形になった．この両種で見られた先体の伸長と扁平化は食虫目の動物種でも認められており，この特徴は翼手目と食虫目の近縁性を反映するものだと推測された．

ICR系由来の遺伝性腎疾患モデルマウスであるICGN系マウスは，幼若期から蛋白尿を示し，細胞外マトリックスの蓄積や腎性貧血など様々な腎障害を呈する．本研究ではBrdU標識法およびTUNEL法を用いてICGN系マウスの腎における増殖細胞とアポトーシス細胞の局在を調べた．健常対照のICR系マウスの腎ではいずれの細胞もほとんど検出されなかったが，ICGN系マウスの腎では両者とも高頻度に検出され，かつこれらの局在が特徴的であった．すなわち，遠位尿細管から集合管への移行部では，全く細胞死を伴わない細胞増殖が認められ，これは腎機能低下に応じた代償性増殖と考えられる．尿細管間質部では，高頻度に両者を検出したが，増殖は線維芽様細胞に，アポトーシスは大きな円形の核を持つ細胞に認められた．このアポトーシス細胞はエリスロポイエチン産生細胞と考えられ，ICGNマウスの腎性貧血に深く関与するものと推測される．

細　菌　学：

*M. avium*感染症豚について，播種性（DI）群とリンパ節限局性（LI）群とに分けてリンパ球幼弱化反応を行ったところ，DI群のCon A，PHAに対する反応性は，対照群及びLI群と比較して有意（Con A $p<0.01$，PHA $p<0.05$）に低下していた．しかしながら，PPDに対しては逆に高い反応を示す個体が多かったことから，DI群でみられた低応答性はT細胞が全体的に反応を抑制された結果ではないと考えられた．

動物行動学：

雌マウスにおけるフェロモンの記憶は交尾刺激を引き金として副嗅球の働きで形成される．すでに，GABA拮抗薬の副嗅球内注入が交尾刺激なしで記憶形成へと導くこと，しかしこの記憶はフェロモン特異性を欠いていることなどが判明している．今回，GABA作動薬であるmuscimolの副嗅球内注入が記憶障害をもたらすか否かを検討した．1～1,000 pmolの用量を注入したが，記憶障害は起こらなかった．複雑な副嗅球内微小回路の存在が推察された．

免　疫　学：

細菌感染に対する宿主防御に果たすマスト細胞の役割が注目されている．成熟したマスト細胞は，通常組織に固着し運動性を有さないが，局所で活性化されると脱分化し，増殖・遊走能を回帰する．本研究では，LPSがマスト細胞のMMP-9産生を増強することを報告する．この新知見は，炎症性細胞浸潤に寄与するMMP-9が，菌体成分の刺激でその産生を亢進し，病変部へのマスト細胞の集積にも関与する可能性を示唆している．