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VAGINOCERVICAL STIMULATION ENHANCES SOCIAL RECOGNITION MEMORY IN RATS VIA OXYTOCIN RELEASE IN THE OLFACTORY BULB

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Abstract—The ability of vaginocervical stimulation (VCS) to promote olfactory social recognition memory at different stages of the ovarian cycle was investigated in female rats. A juvenile social recognition paradigm was used and memory retention tested at 30 and 300 min after an adult was exposed to a juvenile during three 4-min trials. Results showed that an intact social recognition memory was present at 30 min in animals with or without VCS and at all stages of the estrus cycle. However, whereas no animals in any stage of the estrus cycle showed retention of the specific recognition memory at 300 min, those in the proestrus/estrus phase that received VCS 10 min before the trial started did. *In vivo* microdialysis studies showed that there was a significant release of oxytocin after VCS in the olfactory bulb during proestrus. There was also increased oxytocin immunoreactivity within the olfactory bulb after VCS in proestrus animals compared with diestrus ones. Furthermore, when animals received an infusion of an oxytocin antagonist directly into the olfactory bulb, or a systemic administration of α or β noradrenaline-antagonists, they failed to show evidence for maintenance of a selective olfactory recognition memory at 300 min. Animals with vagus or pelvic nerve section also showed no memory retention when tested after 300 min. These results suggest that VCS releases oxytocin in the olfactory bulb to enhance the social recognition memory and that this may be due to modulatory actions on noradrenaline release. The vagus and pelvic nerves are responsible for carrying the information from the pelvic area to the CNS.

Key words: menstrual cycle, VCS, vagus nerve, olfactory system, memory, noradrenaline-antagonist.

Social recognition is the basis upon which all social relationships are built. In rodents, enduring social memories are a component of a variety of complex social and reproductive processes, including pair bond formation in monogamous species (Demas et al., 1997) and selective pregnancy termination in mice (Kaba et al., 1989; Keverne, 1998). Rats and mice also display short-term memories of recently encountered individuals which in rats decay quite rapidly in $<$1 h (Gheusi et al., 1994; Popik and van Ree, 1998).

Social recognition is mainly based upon chemosensory cues in rodents (Sawyer et al., 1984). Olfaction is clearly of primary importance since the removal of the olfactory bulbs (OB) in the brain completely blocks social recognition memory (Dantzer et al., 1990). In the laboratory, social recognition memory is measured by a decline in the amount of time spent investigating the same individual during repeated pairings (Thor and Holloway, 1981).

Mechanical stimulation of the vagina and cervix (VCS) produces morphological and electrophysiological changes in a number of brain regions in the rat controlling reproductive behaviors (Haskins and Moss, 1983; Lee and Erskine, 1996). We have previously found that VCS increases mitral cell activity in the main OB of estrus female rats and decreases it in diestrus animals (Guevara-Guzmán et al., 1997). In the OB, VCS-induced changes are important for olfactory memory formation underlying recognition of offspring in sheep (Kendrick et al., 1987; Kendrick, 2000) and in the accessory OB of female mice for the recognition of male pheromones following mating (Brennan et al., 1990; Kaba and Nakanishi, 1995). In addition, artificial vaginocervical stimulation has been shown to stimulate a rapid onset of maternal behavior in intact multiparous rats primed with estrogen (Yeo and Keverne, 1986). Such genital stimulation has long been known to release oxytocin, which further promotes uterine contractions (Tindal, 1974). The hypothalamic paraventricular nucleus sends oxytocinergic (OT) fibers to many extra-hypothalamic brain areas, including the OB (Sawchenko and Swanson, 1982), and in the sheep when VCS induces maternal behavior and offspring bonding there is also increased oxytocin release in the OB (Kendrick et al., 1988a; Kendrick et al., 1988b). Oxytocin infusions into the OB also influenced maternal responses toward lambs (Kendrick et al., 1997a). Yu et al., 1996, demonstrating that intracranial oxytocin infusions decreased spontaneous firing rate of mitral cells and increased the firing rate of granule cells. The latency of this oxytocin effect on OB cells was around 30 s and the most likely mechanism is that oxytocin excites granule cells, which in turn inhibit mitral cells via their reciprocal dendrodendritic synapses (Shepherd and Greet, 1979).

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Abbreviations: IEI, inter-exposure interval; OB, olfactory bulb; OT, oxytocinergic; VCS, stimulation of the vagina and cervix.

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Memory formation involves plasticity changes in the dendrodendritic synapses between the mitral and granule cells in the OB (Kendrick et al., 1992, 1997b; Brennan et al., 1995). These plasticity changes involve VCS-induced activation of centrifugal noradrenergic inputs to the OB and accessory OB from the brainstem (Rosser and Keverne, 1985; Lévy et al., 1993; Brennan et al., 1995). Oxytocin is also known to facilitate noradrenaline release within the OB and social recognition memory (Kendrick, 2000). We have also demonstrated that release of classical neurotransmitters in the OB after VCS during proestrus or estrus is reduced by pelvic or vagus nerve section (Guevara-Guzmán et al., 2000) so there is a good understanding of the pathways involved all the way from the vagina and cervix to brain olfactory processing regions.

Overall, previous results suggest that sex hormones acting primarily at the level of the OB dramatically enhance the ability of VCS to evoke release of classical transmitters and oxytocin. This fact could be important for mediating plasticity changes underlying olfactory recognition, both in the context of mate and offspring recognition in rodents and other species.

In the current study, we have therefore investigated whether VCS during periods of appropriate sex hormone priming affects the efficacy of social recognition memory in female rats; if the process is oxytocin and noradrenaline dependent and whether effects are dependent upon intact vagus and pelvic nerves to conduct information from the pelvic area to the CNS.

EXPERIMENTAL PROCEDURES

All procedures were in strict accordance with the guidelines and requirements of the World Medical Association Declaration of Helsinki and those of the Ethical Committee of the Faculty of Medicine at the Universidad Nacional Autónoma de México (UNAM).

Animals

Adult female Wistar rats (280–320 g body weight) were used as subjects in these experiments. The animals were caged (28×37×15 cm) in groups of three to four with free access to food and water and were maintained under an artificial light/dark cycle (12-h; lights on at 08:00 h). Two weeks prior to the experimental session, vaginal smears were taken daily in order to identify the precise phase of the estrus cycle the animals were the day of the test. Only regularly cycling animals were used for the test.

Social recognition memory test

This test was based upon a comparison of behavior, particularly investigation duration, between two exposures of the same individual to a novel juvenile animal, after a variable inter-exposure interval (IEI). The social recognition procedure used was similar to that described previously (Reyes-Guerrero et al., 2006; Thor and Holloway, 1981; Vazquez-Garcia et al., 2004). Briefly, 22-day-old female juveniles were used as social stimuli and tests together with the adult subject were conducted in a cage (50×50×42 cm). Each testing session consisted of a sequence of three 4-min trials. The first trial was a habituation period of the adult rat to the test cage; the second trial was the first encounter between the adult rat and the juvenile rat, i.e. the first interaction in the social recognition test; the third trial was the re-exposure to the same or to an unfamiliar juvenile stimulus animal after an IEI of 30 or 300 min. There was a period of 4 min separating these final two tests with the unfamiliar and familiar juvenile. Following each test the test cage was thoroughly cleaned. None of the juvenile stimulus animals were used more than once.

Video recording of investigatory behavior was used to assess the time spent by adult rats investigating the stimulus animal in the social recognition memory test. The data collected from video-recordings were transferred to an IBM-PC computer for off-line analysis. Behaviors considered to be related to social recognition learning and memory were anogenital sniffing, close following, and pawing of the stimulus animal. A selective recognition memory was considered to be present if there was firstly a significant reduction of the mean duration in the performance of these behaviors between the first two encounters with the stimulus juvenile and, secondly, if there was also a significantly longer duration of investigation of the novel juvenile in the third encounter compared with that for the familiar juvenile in the second encounter.

Vaginocervical stimulation

Vaginocervical distension (VCS) was carried out using a (pediatric) urinary latex catheter (different calibers) that was inserted into the vagina. The experiments were conducted between 12:00–16:00 h. Table 1 shows the effect of different calibers used in the social recognition memory. The VCS was administered 10 min before beginning the social recognition memory test and the catheter remained in the vagina for 10 min. Control animals did not receive any VCS.

Immunohistochemistry for oxytocin

Ten minutes after VCS the animals were anesthetized and perfused for immunohistochemistry for oxytocin. Saggital sections of the OB were cut at 5 μm. They were deparaffinized, pre-treated with a heat retrieval solution (Biocare Medical, Concord, CA, USA) and placed in an electric pressure cooker (Decloaking Chamber, Biocare Medical) for 5 min. Immunohistochemistry was performed as previously described (Rivas-Arancibia et al., 2000) following the antibody supplier's protocol (UltraTek HRP-antimonovalent DAB Staining System from SciTek Laboratories, Inc., Logan, UT, USA). The oxytocin antibody (mouse monoclonal antibody; MAB 5296 Chemicon) was diluted 1:100. Sections were then counterstained with a hematoxylin buffer solution. Representative brain sections from each group (non-VCS and VCS-experimental group), were processed in parallel.

Table 1. Effect of VCS on social recognition memory with an IEI of 300 min using different catheter calibers in female rats during proestrus phase of estrus cycle

n=	VCS caliber	1st Exposure	2nd Exposure	3rd Exposure
10	Non VCS	39.7±12.8	22.8±4.3	29.2±6.6
9	8	30.3±2.6	14.1±2.6**	19.6±2.8
9	10	21.7±2.8	13.3±2.1*	20.9±3.1
10	12	26.1±3.2	13.9±2.7**	22.9±2.8#
8	14	22.5±2.2	11.2±1.4***	17.6±1.5
9	16	24.7±1.9	13.9±1.5**	18.8±2.3

Time spent (in seconds) by the adult in investigating conspecific juveniles during each encounter.

* $P<0.05$ between 1st exposure vs. 2nd exposure.

** $P<0.001$ between 1st exposure vs. 2nd exposure.

*** $P<0.0001$ between 1st exposure vs. 2nd exposure.

# $P<0.05$ between 2nd exposure vs. 3rd exposure (Tukey).