Acute Progesterone Treatment Impairs Spatial Working Memory in Intact Male and Female Rats

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Introduction: The aim of this study was to determine if progesterone affects spatial and non-spatial working memory in intact male and female rats.

Methods: Rats received subcutaneous injections of progesterone (500 μg) or vehicle (sesame oil). Four hours after hormone treatments, spatial and non-spatial memories were tested using novel object recognition and spatial object recognition tasks.

Results: Vehicle-treated female rats had higher progesterone serum levels than males, but progesterone treatment produced equivalent progesterone serum levels in both sexes. In the object recognition task—a non-spatial memory task—females showed better performance than males, and progesterone had no effect on either sex. However, in the object replacement task—a spatial memory task—progesterone significantly impaired the retention in both male and female rats as compared with vehicle-treated groups.

Conclusion: These results suggest that acute progesterone treatment interferes with spatial working memory consolidation, but not recognition (non-spatial) working memory. As such, the observed sexual incongruities in progesterone's effects on working memory suggest that progesterone-based hormone therapies have a negative impact on cognition. (*Ethn Dis*. 2010;20[Suppl 1]:S1-83–S1-87)

Key Words: Object Recognition, Spatial Memory, Non-spatial Memory, Gender Differences, Sex Differences

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Introduction

Accumulating evidence suggests that gonadal hormones may in part contribute to sexual dimorphism in learning and memory. For instance, gonadectomy differently affects spatial and non-spatial learning and memory processing in the two sexes.[1–3] During the proestrus stage with its elevated estrogen and progesterone levels, intact female rats more efficiently perform spatial and cued versions of Morris water maze.[4] Progesterone has been implicated, indirectly, in memory formation. For example, during the mid-luteal phase, women showed decreased mental rotation and improved motor skill and fluency.[14] In female rats, the finding that allopregnanolone (a progesterone metabolite [ALLOP]) inhibits learning in the Morris water maze indirectly suggests that progesterone affects memory.[5] Removal of elevated progesterone levels during the female estrous cycle induced cognitive enhancement by improving spatial memory.[6] However, progesterone-replacement in ovariectomized (OVX) rats produces inconsistent results; different studies have shown that progesterone enhances, has no effect, or attenuates working memory. For example, in male and OVX female rodents, footshock avoidance learning and spatial memory were impaired by exogenous administration of progesterone or ALLOP.[5,7] After ovariectomy, estrogen's enhancing effects on spatial tasks were attenuated by progesterone.[8] In young female rats, by contrast, acute pre- or post-training progesterone injection in OVX rodents enhanced the performance of spatial and object recognition tasks.[9–11]

In humans, long-term use of estrogen is combined with progestins—to suppress the occurrence of uterine endometrial cancer or other cancers of the reproductive organs as well as other complications of hormone replacement therapies (HRT). Long-term use of progestins has been associated with memory impairment.[12] Shumaker et al demonstrated that the rate of dementia was doubled in women aged 65 years or older who took estrogen-progestin therapy as compared with women who did not.[13] In rats, progesterone treatment in aged-sham and aged-OVX rats compromised working and reference memory components, and progesterone-treated rats made more working memory errors than vehicle-treated controls.[14] Few studies have looked at progesterone's effect on cognitive performance in an intact—non-stress—and young animal model. Furthermore, little is known about possible sex differences in progesterone's effects on spatial and non-spatial working memory. The aim of the present study was to evaluate the effect of progesterone on spatial and non-spatial working memory by using the object recognition task and to determine whether progesterone's effects are sexually dimorphic.

Methods

Animals

Sixty-day-old intact male and female Fischer rats (Charles River, Raleigh, NC) were individually housed in Plexiglas chambers (20 × 20 × 41 cm) with free access to food and water on a 12-hour light/dark cycle (lights on at 9:00 am). Animal care and use was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and

approved by the Institutional Animal Care and Use Committee of Hunter College.

Object Recognition/Placement Memory Tasks and Progesterone Administration

Memory tasks used to assess object recognition and placement memory were described in previous studies.[3] Briefly, trials consisted of a sample trial (T_1) and a recognition/retention trial (T_2) with increasing intertrial delay intervals. In T_1, two identical objects were placed at one end of an open field and the amount of time spent exploring the two objects was recorded for 3 min. For object recognition memory, one of the identical objects was replaced by a new object during the recognition trial (T_2), and the time spent exploring the old (familiar) and the new (novel) objects was recorded for 3 min. Exploration was defined as the subjects sniffing, whisking or looking at the object from no more than 2 cm away. For object placement testing, one object was moved to a new location during the recognition/retention trial, T_2, and the times spent exploring the object at the old and at the new locations were recorded for 3 min. The objects used for object recognition trials were various bottles or cans. The objects used for object placement recognition trials (eg, can-holders) were more intricately designed and complex than objects used in object recognition trials. The novel objects were counterbalanced among experimental groups. The objects and field were cleaned with disinfectant spray after each subject's trial. To acclimate animals to the field and the task, they received an open field trial for 5 min—this was done to minimize possible stress-mediated responses that might confound the results. Subjects were exposed first to an object placement task with intertrial delay intervals of 1, 10, 60, and 120 min and then to an object recognition task with intertrial delay intervals of 10 and 60 min before experiments began. Following acclimation, each animal underwent object recognition or object placement tests separated by 4 days. A 4-h intertrial delay interval was used to test the retention effect by hormone, as previously described.[13] After T_1, each subject immediately received vehicle (sesame oil) or progesterone (500 μg) subcutaneous injections. All behavioral testing was recorded for later analysis by a blinded observer.

Serum Level Determinations

After the last testing of the day (5 hours after progesterone treatment), animals were sacrificed by decapitation after a brief (20 sec) exposure to CO_2. Trunk blood was collected and centrifuged at 3000 rpm for 20 min at 4°C. Serum was stored at −80°C until use. Samples were analyzed with a Count A Count for progesterone (Diagnostic Product Corporation, LA, CA). Intra-assay coefficient of variance averaged less than 10%. Results were determined using a log-logit analysis within GraphPad Prisms (Graph Pad Software, CA).

Statistics

Data were analyzed as time (in seconds) spent exploring an object or a place. Data were also normalized to the discriminative ratio (time spent on a new object or new position divided by total time), which represents the portion of time exploring the new object or place. To assess the effect of hormone administration on spatial and non-spatial memory, ANOVAs were used (hormone [vehicle, progesterone] × sex [male, female]). To determine the significance of differences between hormone-treatment groups and their respective control vehicle-treatment groups, dependent measure *t*-test analyses were performed. ANOVAs were used to determine the differences in effects on serum levels of progesterone between male and female rats receiving progesterone treatment. When significant interactions were obtained, a Newman-Keuls post hoc test was used to assess the differences among treatment groups. Significance was at the .05 level for all comparisons.

RESULTS

Acute progesterone treatment significantly elevated progesterone serum levels in both female and male rats (Figure 1). Two-way ANOVA revealed significant treatment and sex effect ($F(1, 20) = 69.02$, $P<.001$, and $F(1, 20)=5.92$, $P<.05$, respectively); namely, vehicle-treated females had higher progesterone serum levels than did vehicle-treated males, and after hormone treatment both sexes had higher progesterone levels than their respective vehicle-treated controls ($P<.05$ for all comparisons).

During T_1 of the object recognition task, analysis revealed no differences among groups (8.0 ± 5.8, 9.9 ± 4.1, 8.7 ± 6.6, and 6.3 ± 3.7 sec for vehicle/female, vehicle/male, progesterone/female, and progesterone/male groups, respectively). Further ANOVAs also showed there was no significant difference between T_1 and T_2 within each group ($P>.05$ for all groups), indicating that neither progesterone nor vehicle injections altered the exploration time in the object recognition task. As shown in Figure 2A, regardless of treatment, both male and female rats spent more time exploring new objects than old objects ($t(7)=3.64$, $P<.01$; $t(7)=2.87$, $P<.05$; $t(7)=2.93$, $P<.05$; $t(7)=2.51$, $P<.05$; for vehicle/female, progesterone/female, vehicle/male, and progesterone/male, respectively). For the discriminative ratio, two-way (sex × treatment) ANOVA showed a significant main effect of sex ($F(1, 28)=7.10$, $P<.05$), but a post-hoc test did not reveal any differences among groups (Figure 2B). Taken together, the findings show progesterone treatment did not affect memory consolidation in object recognition learning.

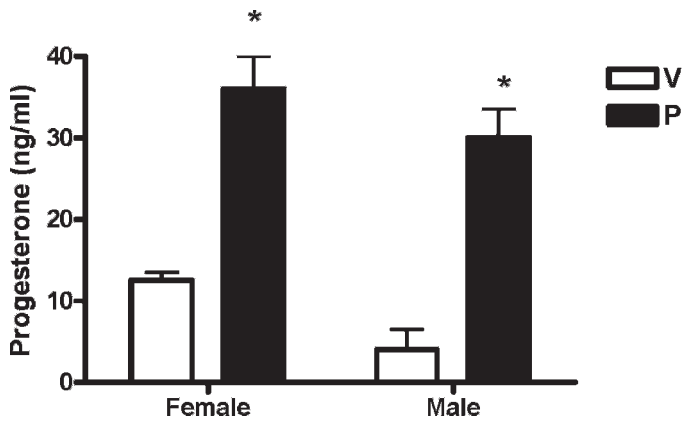

Fig 1. Serum levels of progesterone after acute progesterone administration in male and female rats. Data are presented as the mean (±SEM). * Represents statistically significant differences between progesterone (P)- and vehicle (V)-treated rats. *n*=10 rats per group(by ANOVAS)

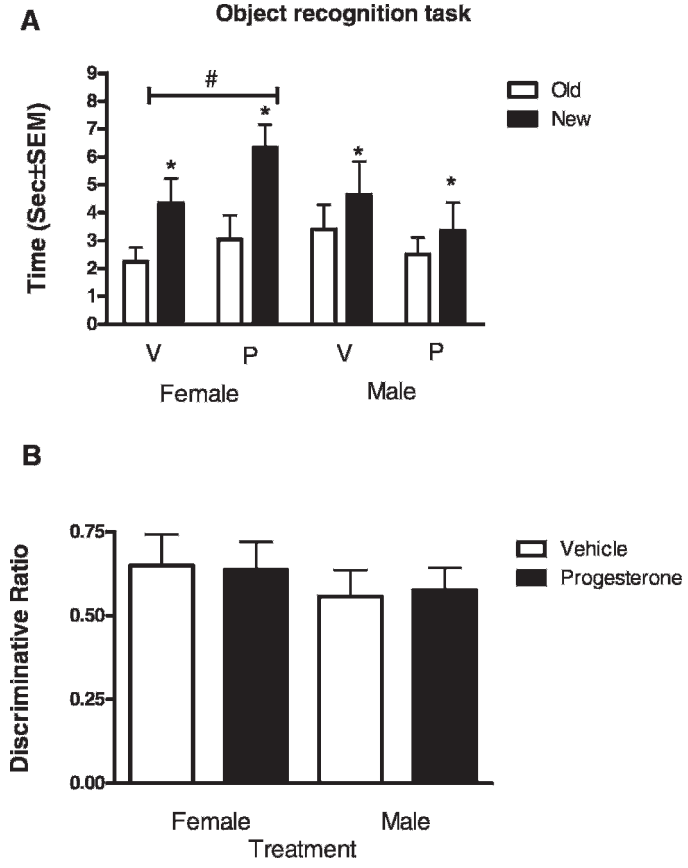

Fig 2. Effects of acute progesterone administration on object recognition of male and female rats. (A) Y-axis represent the amount of time spent with a new (black bar) or an old (white bar) object. (B) Represents the discriminative ratio. Data are presented as the mean ± SEM. *n*=10 per group. * Represents significant differences between the time-spent with the old object vs the new object within each treatment groups (by t-test). #Represents sex interactions; females spent overall more time (by ANOVAS)

Similar to object recognition findings, there were no differences by groups in T_1 of the object placement task (8.5 ± 7.0, 12.7 ± 6.1, 13.0 ± 9.5, and 10.1 ± 4.7 sec for vehicle/female, vehicle/male, progesterone/female, and progesterone/male groups, respectively). Paired *t*-tests also showed there was no statistically significant difference between T_1 and T_2 within each group ($P>.05$ for all groups). Further paired *t*-tests indicated both male and female control rats spent more time exploring an object in a new place than the old place ($t(7)=2.66$, $P<.05$; $t(7)=2.40$, $P<.05$,respectively; Figure 3A). However, progesterone-treated male and female rats did not show significant preference for the new place ($P>.05$ for both groups). For the discriminative ratio, two-way (sex × treatment) ANOVA showed significant treatment main effect ($F(1,28) = 18.11$, $P<.001$). Furthermore, post-hoc testing revealed that the progesterone/male group had a lower discriminative ratio than the vehicle/male group ($P<.01$). Similarly, the progesterone/female group had a lower ratio than their control group ($P<.05$; Figure 3B). Taken together, the results indicated that progesterone impaired spatial working memory consolidation.

DISCUSSION

The results reported here are novel in showing that progesterone affected the subject's ability to discriminate the spatial location of an object, a finding that suggests progesterone inhibits spatial memory formation in male and female rats. This outcome is consistent with Bimonte-Nelson et al,[14] who demonstrated that progesterone was detrimental in different tasks aimed at studying the working and reference memory components in female rats. However, progesterone administration in OVX rats has been shown variously to enhance, have no effect, or diminish

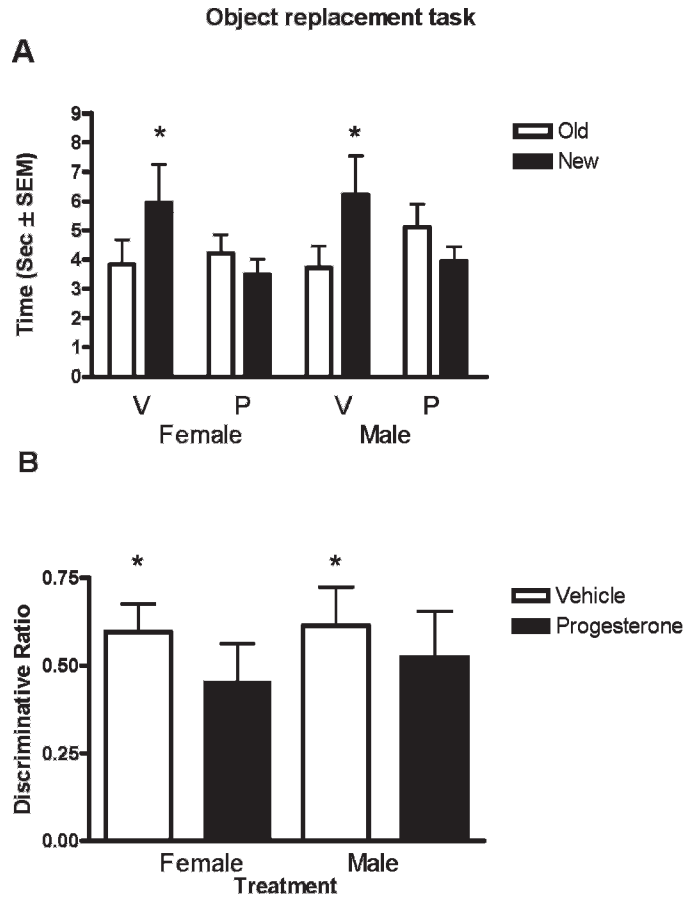

Fig 3. Effects of acute progesterone administration on spatial memory formation in male and female rats. (A) Y-axis represents the amount of time spent with a familiar object in an old (white bar) or a new (black bar) position. (B) Represents the discriminative ratio. Data are presented as the mean ± SEM. *n*=10 per group. * Represents significant differences between the time-spent with the object in the old and new positions within each treatment group (by *t*-test)

the formation of working memory. It has been suggested that experimental variants such as mode of progesterone administration, behavioral task used, age of subject or time after hormone treatment may potentially contribute to these divergences in progesterone effects.[5,14,15] Since most studies do not measure final serum levels of progesterone, it is difficult to relate our finding to that of others. However, our testing model was aimed at addressing progesterone's effects on subjects with intact gonadal and adrenal functions, given that both glands are sources for progesterone release. The timing of progesterone administration may be a key factor in determining the final outcome of the hormone's effects on memory formation and /or recall. For example, progesterone administration before the presentation of the initial trial (T_1 period) may interfere with the consolidation process, whereas administration before the T_2 period may affect the recall process. This issue needs further study.

Clinically used progestins are commonly part of HRT designed to maintain neurological health and function throughout the menopausal transition.[6] Recently the combination of estrogen and progesterone has been suggested to have clinical potential as a protective agent against such disorders as Alzheimer's disease, stroke, and traumatic brain injury.[17] Our study, however, suggests that progesterone treatment (alone) may have a detrimental effect on cognition.

At the used concentration, progesterone has limited effects on motor responses of females[17] and males (Russo et al, *Ethn Dis*, 2010;20[Suppl1]), and so progesterone's effects on memory formation or acquisition may not be related to its motor effects. However, in addition to classic actions at intracellular progestin receptors and membrane-mediated actions of progesterone, some of progesterone's effects could be through actions of its metabolite ALLOP. Both progesterone and ALLOP have potent positive allosteric modulators of $GABA_A$ receptors.[18,19] Thus, as suggested by Harburger et al,[8] progesterone or ALLOP reduction of neuronal activity by activation of GABA receptors may produce analgesic or anxiolytic effects, thereby weakening cognitive function. It is yet to be determined if progesterone's effects are exclusively through altering learning and memory formation and/or recall or through reduction of anxiety or through other non-learning mechanisms.

IMPLICATIONS FOR IMPROVING HEALTH DISPARITIES

Even though progesterone is commonly used for HRT, the understanding of its effects on cognition is limited. Our study demonstrated that progesterone might be detrimental to some aspects of cognitive performance, a finding that suggests the need for further study of whether progesterone alters memory processes in young women taking progesterone-based contraceptives or post-menopausal women using HRT.

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