

Human chorionic gonadotropin (a luteinizing hormone homologue) decreases spatial memory and increases brain amyloid- β levels in female rats

Anne Berry^a, Yasushi Tomidokoro^c, Jorge Ghiso^c, Jan Thornton^{a,b,*}

^a Neuroscience Department, Oberlin College, 119 Woodland Street, Oberlin, OH 44074, USA

^b Biology Department, Oberlin College, 119 Woodland Street, Oberlin, OH 44074, USA

^c Department of Pathology, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA

Received 25 July 2007; revised 3 February 2008; accepted 11 February 2008

Available online 10 March 2008

Abstract

Numerous studies have suggested that estradiol (E) improves spatial memory as female rats with E perform better than those without E. However there is an inverse relationship between E and luteinizing hormone (LH) levels and LH could play a role. We examined whether treatment with the LH homologue human chorionic gonadotropin (hCG), would impair spatial memory of adult E-treated female rats. In the object location memory task, ovariectomized (ovxed) rats treated with E and either a single high dose (400 IU/kg) or a lower repeated dose of hCG (75 IU/kg hourly for 8 h) showed spatial memory disruption compared to ovxed rats treated with estradiol alone. Impairment was attributed to memory disruption as performance improved with shortened delay between task exposure and testing. Tests on another spatial memory task, the Barnes maze, confirmed that hCG (400 IU/kg) can impair memory: although E+veh treated animals made significantly fewer hole errors across time, E+hCG-treated did not. In humans, high LH levels have been correlated with Alzheimer's disease (AD). Because brain amyloid-beta ($A\beta$) species have been implicated as a toxic factor thought to cause memory loss in AD, we analyzed whether hCG-treated animals had increased $A\beta$ levels. Levels of $A\beta$ from whole brains or hippocampi were assessed by Western blot. hCG treatment to E-implanted females significantly increased soluble $A\beta_{40}$ and $A\beta_{42}$ levels. These results indicate that high levels of LH/hCG can impair spatial memory, and an increase in brain $A\beta$ species may account for the memory impairment in hCG-treated rats.

© 2008 Elsevier Inc. All rights reserved.

Keywords: Memory; Spatial memory; Luteinizing hormone; Amyloid- β ; Estradiol; Hippocampus; Alzheimer's

Introduction

Numerous studies have suggested that estradiol (E) is needed for optimal levels of spatial memory by females, particularly on tasks that depend on working memory (for review see [Dohanich 2002](#)). For example, ovariectomy (ovx) decreases spatial memory, and ovxed female rats treated with E perform better than ovxed females treated with vehicle on a number of spatial memory tasks that utilize working memory including the Morris water maze, object place memory task, and the radial arm maze ([Bimonte and Denenberg, 1999](#); [Gibbs, 1999](#); [Luine et al., 1998](#); [Luine et al, 2003](#); [Packard, 1998](#); [Sandstrom and Williams, 2001](#); [Simpkins et al., 1997](#)).

Although E has been considered responsible for these spatial memory effects, there is an inverse relationship between estradiol and the level of luteinizing hormone (LH) and it could be LH rather than E that is responsible. LH is secreted from the anterior pituitary and E normally exerts negative feedback inhibition of LH release. Removal of E by ovx causes a subsequent increase in serum LH and replacement with estradiol lowers LH ([Freeman et al., 1976](#); [Wise and Ratner, 1980](#)). The role of LH in ovxed animals has not been addressed in studies that have examined E's effect on spatial memory. If LH does play a role then high LH in the presence of high E should block E's effects on spatial memory and low levels of LH in the presence of low E should still facilitate spatial memory.

There is evidence that LH can act at the hippocampus to affect behavior. LH and its homologue human chorionic gonadotropin (hCG) can cross the blood-brain barrier ([Knowles, 1972](#); [Lukacs et al., 1995](#); [Oliver et al., 1977](#)) and the highest density of LH/

* Corresponding author. Neuroscience Department, Oberlin College, 119 Woodland Street, Oberlin, OH 44074-1097, USA. Fax: +1 440 7755397.

E-mail address: Jan.Thornton@oberlin.edu (J. Thornton).

hCG receptors in the central nervous system is found in the hippocampus (Lei et al., 1993), a brain area that is essential for memory. LH can modulate neuronal activity in the hippocampus (Gallo et al., 1972) and can modify hippocampus-associated behaviors in rats such as taste neophobia, locomotion and rearing (Lukacs et al., 1995).

Suggestive of a role for LH in memory, elevated LH has been implicated as a risk factor in Alzheimer's disease (AD). AD is the most common form of clinical dementia and is characterized by selective neurodegeneration in the hippocampus and progressive memory loss leading to cognitive decline. Reports find serum LH to be significantly higher in individuals with AD compared to age matched controls (Bowen et al., 2000; Short et al., 2001; Hogervorst et al., 2004; though for contradictory evidence see Hogervorst et al., 2003). Patients with Down syndrome have elevated levels of LH throughout life and develop cognitive impairment and AD-like lesions early in life (Mann, 1988; Oliver and Holland, 1986). Moreover, immunohistochemical analysis suggests that the amount of LH is increased in the cytoplasm of pyramidal neurons in AD brains (Bowen et al., 2002).

Brain amyloid proteins have been implicated in the pathophysiology of AD and it is possible that LH may increase amyloid- β ($A\beta$) levels. In the hippocampus amyloid protein precursor, a single pass transmembrane protein, is cleaved by secretases into $A\beta_{40}$ and $A\beta_{42}$. Increases in the secretion and aggregation of $A\beta$ molecules are thought to be responsible for cell toxicity and memory impairment in AD (Koh et al., 1990; Yankner et al., 1990; Mattson et al., 1992, 1993; Morgan et al., 2000; Naslund et al., 2000). A series of studies have shown that levels of soluble $A\beta$ correlate with the degree of cognitive impairment and disease progression in animal models and AD subjects (Kuo et al., 1996; McLean et al., 1999; Mucke et al., 2000; Naslund et al., 2000) and increasing evidence has suggested that soluble non-fibrillar $A\beta$ rather than the insoluble fibrillar counterpart is important for the pathophysiology of the disease (Walsh, 1999; Lambert et al., 2000). In addition, LH has been found to cause elevated levels of $A\beta$ in neuroblastoma cells *in vitro* (Bowen et al., 2004), and the prolonged suppression of LH in both normal mice (Bowen et al., 2004) and an AD mouse model (Casadesus et al., 2006) has been shown to decrease $A\beta$ load and aggregates, respectively, *in vivo*.

In the present studies we explored whether elevated levels of LH may contribute to the memory changes previously attributed to E. hCG was used in place of LH due to its availability. Both hCG and LH have a similar structure and act at the same receptor to produce similar effects (Lei and Rao, 2001; Loosfelt et al., 1989; McFarland et al., 1989). More specifically, we examined whether elevated hCG would disrupt E's enhancement of spatial memory in female rats. We then tested whether elevated hCG, at levels that impair spatial memory, would increase $A\beta_{40}$ or $A\beta_{42}$ in the brain.

Materials and methods

Animals

Adult female Sprague–Dawley rats derived from the breeding of animals purchased from Hilltop Animal Laboratories were used. All were weaned at

4 weeks and housed in same-sex groups. Just prior to surgery animals were housed in groups of 3 in plastic cages measuring 27.9 cm \times 20.3 cm \times 17.8 cm. Animals were kept on a 14 hour light: 10 hour dark cycle (7:00 pm lights off) with ad libitum access to Purina Labdiet and water. All behavioral testing took place between 7:30–9:30 pm under red light. All procedures met NIH standards and were approved by the Oberlin College Institutional Animal Care and Use Committee.

Hormones

All rats were ovariectomized (ovx) under isoflurane anesthesia and implanted subcutaneously with a silastic capsule that had been equilibrated in saline overnight prior to implantation. Silastic capsules (15 mm long with an inner diameter of 1.57 mm, and an outer diameter of 3.18 mm), were plugged with wood and sealed on either end with elastomer (Dow Corning) so that each capsule contained either 5 mm of estradiol-17 β (E; Sigma) or no hormone (blank). Because the permeability, diameter, wall thickness, and concentration gradient are constant in the capsule, the length of the capsule determines the diffusion rate (Legan et al., 1975). Estradiol capsules of this size provide constant circulating levels of approximately 75 pg/ml estradiol for over 1 year (Legan et al., 1975; Karsch et al., 1973). Purified hCG (Prospec-Tany) was reconstituted in deionized water (400 IU/ml) and stored in aliquots at -20°C for 1–4 weeks. hCG or water vehicle was injected intraperitoneally.

Open field habituation and anxiety/activity tests

Prior to the object location memory task, females were habituated to the open field arena. The arena was 80 cm \times 80 cm \times 30 cm with the floor marked in a grid comprised of 10 cm \times 10 cm squares. A large black and white extra-maze cue (a white 35 \times 35 cm cross on a black 70 \times 55 cm background) was located on one wall outside of the open field box. On the first day of habituation, groups of 2–4 rats were placed in the arena (with wood shavings) for 20 min. On day 2 of habituation groups of 2–4 rats were placed in the arena for 20 min (without wood shavings). On the third day of habituation, rats were placed individually in the arena (with wood shavings) for 5 min.

To determine anxiety and activity levels, rats were placed individually in the arena (without wood shavings) for 5 min and the number of 10 cm squares crossed (a measure of activity) and the number of seconds spent in the center of the open field arena (a measure of anxiety: rats that are anxious will spend less time in the center of an open field) were recorded. Rats were tested the day after habituation (before hCG or vehicle) and immediately after the first Object Location Memory Test, approximately 7–9 h after hCG or vehicle administration.

Object Location Memory Test

This task is based on one described by Ennaceur et al. (1997). Testing took place in the open field arena described above, covered in wood shavings. Each test consisted of two trials: an exposure trial and a test trial. During the exposure trial, two identical objects were placed in two quadrants of the open field 20 cm from each wall. The rat was introduced into the arena equidistant from the two objects with its head facing the wall and allowed to explore for 5 min. The amount of time the rat explored each object (i.e. the rat's nose was at most 2 cm away from the object) was measured in seconds (Ennaceur and Delacour, 1988). Afterwards the rat was returned to its home cage. Shavings in the open field were mixed to eliminate odor trails and objects were cleaned with 70% ethanol. After a designated intertrial delay (see specific experiments), the rat was returned to the open field for a test trial. For this trial, one object was moved to a new quadrant in one of two counterbalanced configurations, the rat was introduced into the same side of the arena as for the exposure trial and allowed to explore for 3 min and the amount of time each object was explored was recorded. Animals underwent 2–3 behavioral tests, separated by intervals of 4 days. Data from all tests were averaged for each rat. Testers were blind to the treatment group they scored.

Barnes maze

The Barnes maze tested the ability of rats to use fixed spatial cues to locate an escape box that allowed the rats to escape a lighted platform (see Barnes,

1979). Briefly, a raised white platform 1.22 m in diameter contained eighteen holes (9.5 cm in diameter) only one of which allowed access to a removable escape box. The white platform was illuminated by four 200 Watt flood lamps and was encircled by a black curtain. Spatial cues were placed on the inside of the curtain. An upside down container in the middle of the platform was attached to a pulley. A camera was secured near the ceiling and captured the entire circumference of the platform. The output of the camera fed to a TV/VCR visible to the experimenter. To begin a trial, a rat was placed under the container in the center of the maze and the container was lifted. On the first day, the rat was immediately assisted to the escape hole by the experimenter and allowed to remain there for 90 s. This was considered a habituation trial. For the remaining trials, the rat had to locate the escape hole on its own. When the rat entered the escape hole, the hole was covered and the rat remained there for 90 s. Between trials the platform and goal box were cleaned with 40% ethanol to disrupt olfactory cues. For each trial the escape box remained fixed in relation to the spatial cues but the table was rotated a random number of holes in order to prevent cues from the table. Rats were scored for the number of hole errors defined as breaking of the vertical plane of a hole. Rats underwent five trials per day for five consecutive days. Hole errors per trial were averaged each day for each animal.

Experimental procedures

Experiment 1a: Object Location Memory Test following single hCG injection

Eighteen adult rats (280–350 g) were ovxed and implanted with either an estradiol-filled or blank capsule. Four weeks later the animals were habituated to the open field. The next day, animals with an E capsule were either injected with 400 IU/kg hCG (E+hCG group, $n=6$) or with the hCG vehicle (E+veh group, $n=6$). Animals with a blank capsule were injected with hCG vehicle (veh+veh group, $n=6$). Six hours after hCG or vehicle injection animals were tested on the Object Location Memory Test with an intertrial interval between exposure and test trials of 30 min. Animals were subsequently tested twice more with 4 days between testing sessions. Animals were also tested for anxiety and activity the day after habituation (before hCG or vehicle) and 7–9 h after the first hCG or vehicle injection. The center of the arena was defined as 60×60 cm. This dose of hCG was chosen based on experiments that have found effects of hCG on several behavioral endpoints using doses of 100–500 IU (e.g. Telegdy and Rozsahegyi, 1971; Lukacs et al., 1995).

Experiment 1b: Barnes maze following single hCG injection

Five days after the last Object Location Memory Test, animals began testing in the Barnes maze. Animals were tested five consecutive days and were injected with either hCG (400 IU/kg) or vehicle 6 h before testing each day. One animal from the E+veh treated group did not look for the escape hole and was eliminated from the data analysis.

At the conclusion of the experiment it was verified that the estradiol implants were still functional. Estrogens produce cornification of cells in the vagina of rats (Jones et al., 1961). The presence of estradiol in rats treated with estradiol-filled implants was confirmed through vaginal smears which showed predominately cornified cells for seven consecutive days. Rats treated with blank implants showed predominately leukocytes for seven consecutive days. Female rats have high counts of leukocytes in the vagina when estradiol is absent (Brenot et al., 1952).

Experiment 2: Object Location Memory Test following single hCG injection with Varying intertrial intervals.

Eighteen adult rats (240–350 g) were ovxed and implanted with either an estradiol-filled or blank capsule. One week later they were habituated to the open field. Animals with an E capsule were either injected with 400 IU/kg hCG (E+hCG group, $n=6$) or with the hCG vehicle (E+veh group, $n=6$). Animals with a blank capsule were injected with hCG vehicle (veh+veh group, $n=6$). Six hours after hCG or vehicle injection animals were tested on the Object Location Memory Test. Each rat was tested with three different intertrial delays between the exposure and test trials: 1 min, 30 min, or 24 h, in random order (for the 24 h delay, animals were injected again 6 h before the test trial). Each animal was tested two times at each intertrial delay for a total of six testing sessions for each animal. Each animal had 4 days between testing sessions. Exploration times for each intertrial delay were averaged for individual rats. Animals were also tested for anxiety and activity the day after habituation

(before hCG or vehicle) and 7–9 h after the first hCG or vehicle injection. To increase the sensitivity of the test, the center of the arena was redefined as $40 \text{ cm} \times 40 \text{ cm}$.

Experiment 3: Object Location Memory Test following multiple lower dose hCG

Eighteen adult rats (225–295 g) were ovxed and implanted with either an estradiol-filled or blank capsule. One week later they were habituated to the open field. The next day, animals with an E capsule were either injected with hCG (75 IU/kg) once an hour for 8 h (E+hCG group, $n=6$) or with the hCG vehicle hourly for 8 h (E+veh group, $n=6$). Animals with a blank capsule were injected with hCG vehicle hourly for 8 h (veh+veh group, $n=6$). Dr. Jon Levine (Northwestern University) provided the injection protocol based on the information that a dose of 75 IU/kg hCG (i.p.) is sufficient to trigger ovulation in female rats, and normal pulsatile LH secretion occurs in the ovxed rat ~ 1 pulse/30 min. Six hours after the last hCG or vehicle injection animals were tested on the Object Location Memory Test with an intertrial interval between exposure and test trials of 30 min. Each animal was tested twice and the two tests were averaged. Animals also underwent activity/anxiety testing: one test on the day after habituation (prior to hCG or vehicle injection), and one on the first day of memory testing (7–8 h after the last injection of either hCG or vehicle). The center of the arena was defined as $40 \text{ cm} \times 40 \text{ cm}$.

Experiment 4a: analysis of A β levels in whole brains of rats treated with E+hCG

Seven days after the last Object Location Memory Test, E+hCG and E+veh ($n=6$ per group) females from expt 2 were injected with either a high dose of hCG (400 IU/kg) or vehicle, respectively. Six hours later animals were anesthetized with isoflurane and brains were removed and immediately frozen. Tissue was homogenized in ice-cold TBS (20 mM Tris pH 7.4/150 mM NaCl) containing protease inhibitors (1 mM PMSF, 1 μ M pepstatin A, 1 μ M leupeptin) and 10 mM EDTA using a ratio of 5 ml TBS per gram of tissue and a Dounce glass homogenizer immersed on ice. Homogenates were centrifuged for 1 h at 112,000 g using a Beckman Optima XL-100K ultracentrifuge at 4 °C. After the recovery of supernatants, the resulting pellets were subjected to another round of homogenization/centrifugation and both supernatants were combined for each animal. The A β peptides present in each sample were immunoprecipitated with magnetic beads (Dynabeads, Invitrogen) and coated with anti-A β antibody 4G8 (Signet) as described elsewhere (Tomidokoro et al., 2005). The volume of TBS extract used for each immunoprecipitation was equivalent to 500 mg of brain tissue with whole brains weighing 1.6 g–1.9 g. After incubation for 3 h at room temperature with bi-directional mixing, beads were washed three times with 1 ml of 10 mM phosphate buffer, pH 7.4 containing 137 mM NaCl and 2.7 mM KCl (PBS; Sigma). Bound materials were eluted in 10 μ l of Tris–Tricine sample buffer (BioRad) with 5 μ l of 1 M dithiothreitol, separated by 16% Tris–Tricine SDS-PAGE, and electro transferred onto polyvinylidene difluoride membranes (Millipore) using 10 mM 3-cyclohexylamino-1-propanesulfonic acid (CAPS; Sigma) buffer, pH 11 containing 10% (v/v) methanol. After boiling in PBS for 5 min to increase A β immunoreactivity (Tomidokoro et al., 2005), membranes were blocked in 5% skim milk dissolved in PBS containing 0.1% Tween-20 and incubated with the proper primary antibody – either anti-A β 4G8 (Covance), anti-A β 40 or A β 42 (Biosource) – followed by either anti-rabbit or anti-mouse, horseradish peroxidase-labeled F(ab') $_2$ (Amersham GE Healthcare). Synthetic human A β (1–40) and (1–42) as well as rodent A β (1–40) peptides were used as positive controls. Fluorograms were developed with Super Signal (Pierce) and exposed to Hyperfilm ECL (Amersham GE Healthcare). Signal intensity was evaluated by densitometric analysis using Image-J (<http://rsb.info.nih.gov/ij/>). Signal intensities were obtained from duplicate membranes for each sample and averaged.

Experiment 4b: analysis of A β levels in hippocampi of rats treated with E+hCG

A β analysis on isolated hippocampi was done using fourteen adult rats (200–260 g) that were ovxed, implanted with E and given the multiple lower dose of hCG or vehicle injections described in experiment 3. Seven days later females were injected with either a high dose of hCG (400 IU/kg, E+hCG group: $n=7$) or vehicle (E+veh group, $n=7$). Six hours later, brains were removed, and bilateral hippocampi were isolated and frozen. A β molecules were

extracted as described in experiment 4a but samples were pooled within groups. After SDS-PAGE of A β molecules immunoprecipitated using mouse monoclonal 4G8, and A β 4 kDa monomers were detected with the rodent specific mouse monoclonal A β antibody m3.1 (kind gift from Dr. Mathews Center for Dementia Research, Nathan Kline Institute) and visualized as described above, utilizing the same positive controls.

Data analysis

All data are given as mean \pm the standard error (SE). Any analyses that did not reach an alpha level of ≤ 0.05 were considered statistically nonsignificant. For the anxiety and activity tests, number of squares crossed and seconds spent in the center were analyzed using 2-way analysis of variance (ANOVA): 3 experimental groups \times 2 treatments (pre- vs post-injection). For the Object Location Memory Test, total exploration time by the different groups was analyzed with one way ANOVA. Potential bias for one object location during exploration trials was tested using paired *t*-tests. The test trials of the Object Location Memory Test were analyzed using 2-way ANOVA with 3 treatment groups (E+veh, veh+veh, E+hCG) \times 2 objects (moved and unmoved). If significant *F* values were found, then paired *t*-tests on each group tested whether time spent with the moved object was greater than the time spent with the unmoved object. If a group spent significantly more time exploring the moved than the unmoved object, those animals were considered to have demonstrated good spatial memory. Barnes maze data were analyzed with a two way ANOVA (three treatment groups by two test days: days 1 and 5) followed by single degree of freedom comparisons. A β data were analyzed with independent *t*-tests.

Experiment 1a: Object Location Memory Test following a single hCG injection

The Object Location Memory Test was used to assess the effects of hCG on spatial memory in female rats. This task (Ennaceur et al., 1997) measures spatial memory using rats' natural tendency to preferentially explore objects in locations where they have not previously encountered them. That is, rats spend more time exploring a familiar object placed in a novel location than a familiar object placed in a familiar location.

Results. Elevated hCG blocked the effects of estradiol on spatial memory (Fig. 1). A 2-way ANOVA (group \times object) showed no main effect of group ($F(2,15) = .475$, $p = .642$), a main effect of object ($F(2,15) = 12.970$, $p = .003$) and a significant interaction ($F(2,15) = 6.737$, $p = .008$). Subsequent compar-

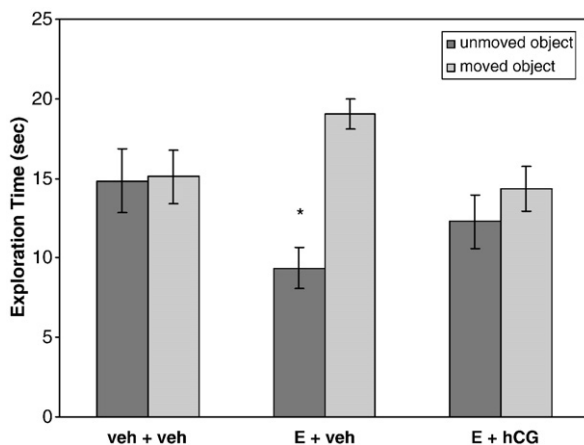


Fig. 1. Single hCG injection suppresses performance on Object Location Memory Test. Ovxed estradiol-treated females (E+veh group) showed robust spatial memory as evidenced by their preferential exploration of the moved object (* $p = 0.001$ compared to nonmoved). Injection of hCG impaired estrogen's enhancement of spatial memory. That is, a single injection of hCG into estradiol-treated rats (E+hCG group) eliminated the preferential exploration of the moved object. hCG-treated rats performed similarly to ovxed females lacking estradiol (veh+veh group) that did not show any preference for the moved object. $N = 6$ per group.

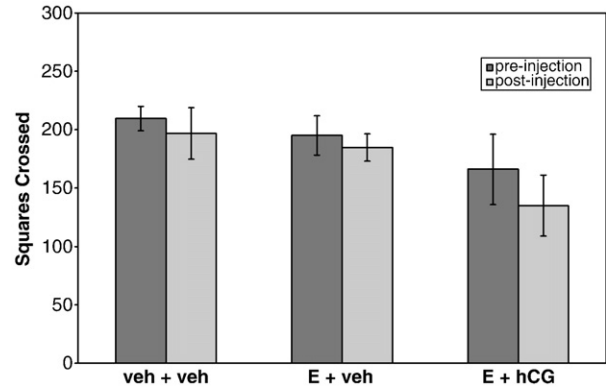


Fig. 2. Single hCG injection does not affect activity levels. Groups did not significantly differ in their levels of activity as measured by the number of squares crossed in an open field arena. There was no statistically significant effect of experimental group (veh+veh, E+veh, vs E+hCG groups) or testing session (pre-injection vs post-injection) and there was no significant interaction. $N = 6$ per group.

isons indicated that the only group that showed robust spatial memory was the ovxed, estrogen-treated females (E+veh group) as evidenced by their preferential exploration of the moved object ($p = 0.001$). In contrast, veh+veh treated rats did not significantly discriminate between moved and unmoved objects. Treatment with hCG blocked estradiol's enhancement of spatial memory performance as E+hCG-treated rats did not significantly discriminate between moved and unmoved objects.

Differences amongst experimental groups during test trials were not due to differential exposure to the objects. During the exposure trials there were no significant differences between experimental groups in total amount of time spent exploring the two objects (E+veh=42.44 \pm 1.14 s, E+hCG=42.89 \pm 2.49, veh+veh=46.67 \pm 4.12). There was also no spatial preference for an object in one location or the other during the exposure trial as there were no statistically significant differences between the amounts of time spent exploring the two objects for any of the treatment groups ($p > 0.2$ for all groups). These results demonstrated that animals were not biased in their initial exploration of the objects.

It is unlikely that these differences in spatial memory performance were due to differences in activity or anxiety levels. Groups showed similar levels of activity and anxiety as measured by the number of squares crossed in an open field arena and the amount of time spent in the center of the arena, respectively. Fig. 2 shows the number of squares crossed for each group before and after hCG or vehicle injection. Although the hCG-treated animals tended (nonsignificantly) to cross fewer squares, this was true both before and after hCG so was not due to hCG treatment. ANOVA revealed no statistically significant effect of experimental group or testing session (pre-injection vs post-injection) and there was no significant interaction. Consistent with this, amount of time spent in the middle of the arena was similar for the three groups (E+veh: pre-injection=28.67 \pm 3.82 s, post-injection=33.5 \pm 2.22 s; E+hCG: pre-injection=25.00 \pm 5.67 s, post-injection=26.83 \pm 10.10 s; veh+veh: pre-injection=29.83 \pm 3.82 s, post-injection=39.5 \pm 4.98 s) and ANOVA revealed no statistically significant effect of experimental group or testing session and there was no significant interaction.

Experiment 1b: Barnes maze following single hCG injection

To determine if the effect of hCG on spatial memory had broader applicability, a second type of spatial memory test, the Barnes maze, was used.

Results. As shown in Fig. 3, hCG blocked estradiol's enhancement of spatial memory in a Barnes maze. ANOVA indicated a significant main effect of day ($F(1,14) = 6.188$, $p = .026$), no main effect of treatment ($F(2,14) = .110$, $p = .896$) and no treatment \times day interaction ($F(2,14) = 1.138$, $p = .348$). As expected, further analysis indicated that estradiol-treated animals improved their spatial memory performance across time as indicated by the significant decrease in the number of errors on test day 5 compared to day 1 ($p = 0.05$). In contrast, there was no significant change in the number of errors across days 1 and 5 shown by the E+hCG-treated animals or the veh+veh group (ANOVA, $p > 0.05$).

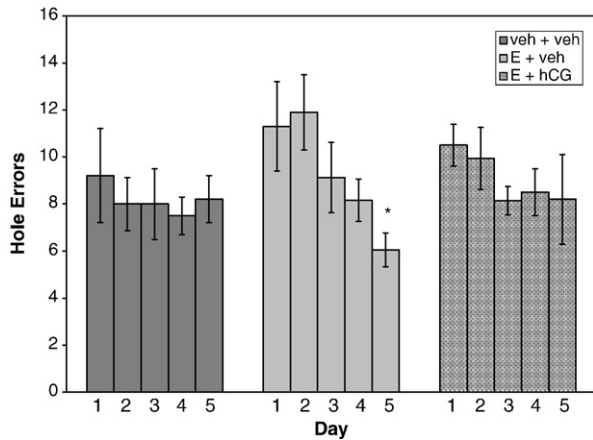


Fig. 3. Single hCG injection suppresses performance in the Barnes maze. Estradiol-treated animals improved their spatial memory performance across time as indicated by the fact that the number of errors made across test days significantly decreased (E+veh group day 1 vs day 5, $*p=0.05$, $n=5$). hCG blocked the effects of estradiol on spatial memory. That is, estradiol+hCG-treated females (E+hCG group) did not improve their spatial memory performance (the number of hole errors did not significantly differ on day 5 compared to day 1, $n=6$). Rats lacking estradiol (veh+veh group) also did not show improvement from day 1 to day 5 ($n=6$).

Experiment 2: Object Location Memory Test following single hCG injection: varying intertrial intervals

To determine that hCG affects spatial memory retention rather than acquisition or sensory responsivity, intertrial intervals were varied. If all treatment groups are able to respond to and learn about the location of objects then all groups should demonstrate learning with a short intertrial delay between exposure and test trials. Moreover, longer intertrial intervals should interfere with the spatial memory of all groups and eliminate any group differences.

Results. All groups showed good spatial memory performance with a 1 min intertrial interval, no group showed intact spatial memory with a 24 hour delay, and only the E+veh group showed good spatial memory with a 30 min intertrial interval (Fig. 4). For the 1 min intertrial interval, a 2-way ANOVA showed a main effect of object $F(1,15)=74.294$, $p<.001$, no effect of group and no interaction. Subsequent analyses indicated that females in all treatment groups spent significantly more time exploring the moved than the unmoved object (veh+veh group $p=0.001$; E+veh group, $p=0.007$; E+hCG group, $p=0.004$). For the 24 h intertrial interval a 2-way ANOVA showed no main effect of group or object and no interaction ($p>0.1$ for all comparisons) indicating that none of the groups showed spatial memory. For the 30 min intertrial interval a 2-way ANOVA showed a main effect of object $F(1,15)=12.985$, $p=.003$, no effect of group and a significant interaction $F(2,15)=5.669$, $p=.015$. In this case, only the estradiol-treated females showed good spatial memory by spending more time exploring the moved vs unmoved object (E+veh group, $p=0.010$). The other groups did not ($p>0.2$).

Consistent with experiment 1a, hCG had no significant effect on activity or anxiety levels as measured by the number of squares crossed in an open field arena (Table 1) or the amount of time spent in the center of the arena, respectively. An ANOVA of the number of squares crossed revealed no statistically significant effect of experimental group or testing session and there was no significant interaction. Consistent with this, amount of time spent in the middle of the arena did not differ significantly for the three groups either before or after injection (E+veh: pre-injection= 11.83 ± 1.74 s, post-injection= 14 ± 1.57 s; E+hCG: pre-injection= 12.33 ± 0.84 s, post-injection= 19.17 ± 2.18 s; veh+veh: pre-injection= 11.83 ± 1.97 s, post-injection= 10.83 ± 2.69 s: ANOVA $p>0.05$ for main effects and interaction).

Experiment 3: Object Location Memory Test following multiple hCG injections

Although experiments 1 and 2 indicated that a single high dose of hCG can block the effects of estradiol on spatial learning, we wanted to examine whether

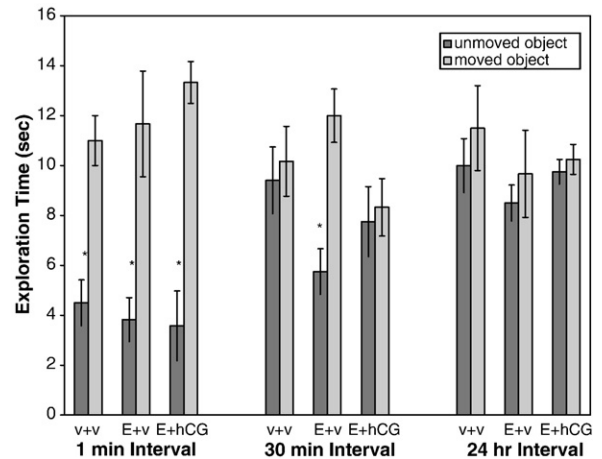


Fig. 4. Single hCG injection and Object Location Memory Test with varying intertrial intervals. All groups showed strong spatial memory with a 1 minute intertrial interval as indicated by a clear tendency to explore a moved object more than an unmoved object ($*p=0.001$ to 0.007). No group showed significant spatial memory at the 24 h interval. Consistent with expt 1a, with a 30 min intertrial interval only the females in the estradiol-treated (E+v) group showed clear spatial memory ($*p=0.010$) and treatment with hCG blocked estradiol's enhancement of spatial memory (E+hCG group). $N=6$ per group.

a regimen that more closely mimics a normal ovariectomized pattern of LH would also impair spatial memory. A multiple injection protocol was developed to model physiological levels of LH that would be seen after ovx in order to evaluate the effect of sustained, physiologically high levels of LH/hCG on spatial memory.

Results. The multiple dose of hCG blocked the effects of estradiol on the enhancement of spatial memory (Fig. 5). A 2-way ANOVA showed no main effect of group $F(2,15)=2.627$, $p=.105$, a main effect of object $F(1,15)=19.446$, $p=.001$, and a significant interaction $F(2,15)=3.362$, $p=.010$. Further analyses showed that ovxed estradiol-treated females (E+veh group) again showed robust spatial memory as they preferentially explored the moved object during the test trials ($p=0.004$). Treatment with hCG blocked estradiol's enhancement of spatial memory performance as rats treated with multiple injections of hCG in the presence of estradiol (E+hCG group) did not significantly discriminate between moved and unmoved objects. Rats lacking estradiol (veh+veh group) also showed impaired spatial memory and did not preferentially explore the moved object ($p>0.05$).

These differences did not appear to be the result of differential exposure to the objects. There were no significant differences between experimental groups in total time spent exploring the two objects during the exposure trial (E+veh= 36.08 ± 3.34 s, E+hCG= 36.50 ± 2.13 s, veh+veh= 34.33 ± 3.42 s;

Table 1
Single hCG injection and activity levels

	Squares Crossed	
	Pre-injection	Post-injection
veh + veh	239.5±19.2	241.5±14.8
E + veh	222.3±5.1	220.5±4.2
E + hCG	236.3±9.1	248.5±7.3

Activity levels with varying intertrial intervals. Using an open field, activity levels were measured just before (pre-injection) and after (post-injection) the first Object Location Memory Test with varying intertrial delays. There were no statistically significant differences in activity levels as measured by the number of squares crossed when comparing experimental groups (veh+veh, E+veh, vs E+hCG) or between pre-injection and post-injection tests for any particular group. $N=6$ per group.

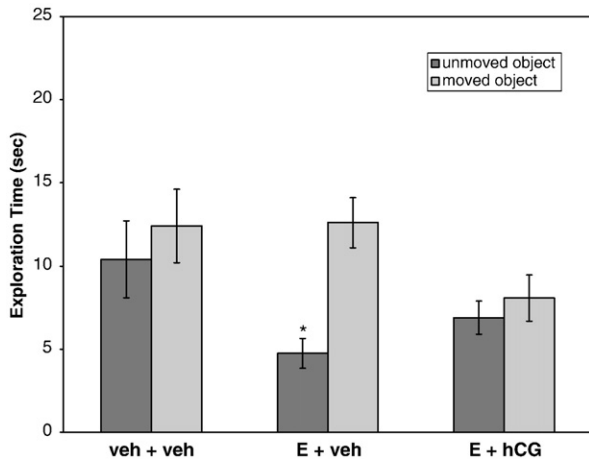


Fig. 5. Multiple hCG injections suppress performance in Object Location Memory Test. A lower, repeated dose of hCG blocked the effects of estradiol on the enhancement of spatial memory. Ovxed estrogen-treated females (E+veh group) showed robust spatial memory as they preferentially explored the moved object (* $p=0.004$). Rats treated with multiple injections of hCG in the presence of estradiol (E+hCG group) showed poor spatial memory as there was no significant difference in exploration times of the moved and unmoved objects. Rats lacking estradiol (veh+veh group) also showed impaired spatial memory and did not preferentially explore the moved object. $N=6$ per group.

ANOVA $p>0.05$) nor was there a preference for one object over the other by any group ($p>0.1$ for all groups).

Groups did not significantly differ from each other in their levels of activity or anxiety as measured by the number of squares crossed or time spent in the middle of an open field arena, respectively. An analysis of squares crossed suggested that the multiple injection regimen may have decreased the activity of all of the animals (ANOVA revealed a significant main effect for testing session (pre-injection vs post-injection $F(1,5)=7.916$, $p=0.013$)), but there was no effect of experimental group ($F(2,15)=0.40$, $p=0.916$), and there was no significant interaction ($F(1,5)=0.882$, $p=0.435$), suggesting that all groups were affected equally. Moreover, groups did not significantly differ in their levels of anxiety as measured by the number of seconds spent in the middle of an open field arena (E+veh: pre-injection = 12.00 ± 0.89 s, post-injection = 8.83 ± 2.66 ; E+hCG: pre-injection = 15.17 ± 2.50 , post-injection = 13.67 ± 4.25 ; veh+veh: pre-injection = 14.33 ± 2.33 , post-injection = 15.00 ± 2.84). ANOVA revealed no significant effect of experimental group or testing session and there was no interaction.

Experiment 4a and 4b: biochemical analysis of A β in rats treated with E+hCG

To determine whether hCG treatment, at a level that affects spatial learning, is capable of increasing the amounts of A β *in vivo*, brain A β levels were assessed by a combination of immunoprecipitation and Western blot analysis.

Results. 4a: Western blot analysis revealed that hCG increased the amount of total dimeric 8 kDa A β and both A β 40 and A β 42 in rat brains. Representative blots of extracted A β are presented in Fig. 6 panels A to C. As shown in panel A, total A β for E+hCG and E+veh treated animals was assessed using 4G8. hCG clearly increased the amount of dimeric A β . To clarify which A β species were increased in hCG-treated animals, A β molecules were labeled by C-terminal specific antibodies. The blots show that both A β species, A β 42 (B) and A β 40 (C), were increased in hCG-treated animals. Densitometric analysis indicated that hCG treatment significantly increased levels of soluble A β 42 dimers ($t(10)=2.225$, $p=0.050$) and A β 40 dimers ($t(10)=2.460$, $p=0.034$) relative to vehicle treatment (Fig. 6). There was a 25% average increase in A β 42 and a 38% average increase in A β 40.

4b: hCG also increased the amount of A β in the hippocampus of rat brains (Fig. 6 panel D). Blots were probed with the antibody m3.1, which labels 4 kDa monomeric A β . Densitometric analysis indicated that hCG treatment increased the level of A β by 24% in the hippocampi of E-implanted animals compared to E-implanted animals treated with vehicle.

Discussion

The present studies demonstrate that elevated levels of the LH homologue hCG can disrupt estradiol's enhancement of spatial memory in female rats and increase brain amyloid- β levels. Ovxed rats implanted with E and treated with hCG either in a single high dose or multiple lower doses performed more poorly on the Object Location Memory Test a measure of spatial memory, than ovxed rats implanted with E and treated with vehicle. hCG-induced spatial memory deficits were replicated using the Barnes maze. Rats treated with memory-impairing levels of hCG showed elevation in brain A β species, A β 40 and A β 42 dimers and A β monomers.

Spatial learning and memory deficits following hCG treatment are responsible for differences in task performance; results do not appear to be due to effects on anxiety or activity. Although a previous study reported decreases in activity and anxiety after hCG treatment of proestrus female rats (Lukacs,

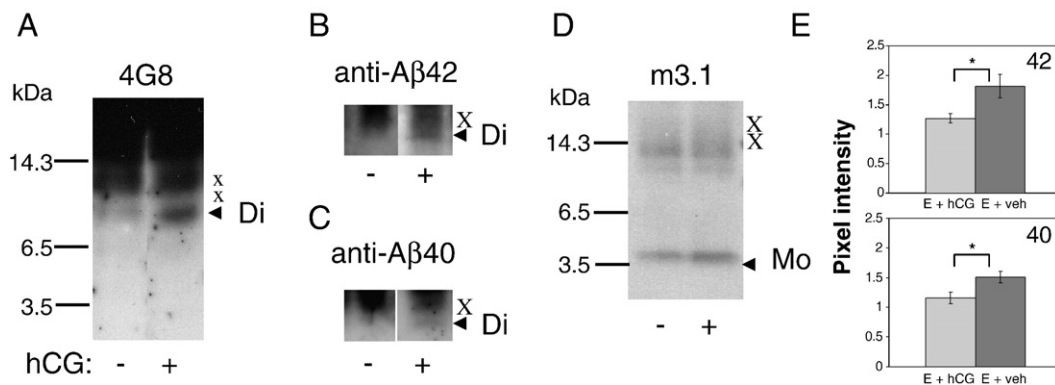


Fig. 6. hCG increases β -amyloid levels in brain. Western blot analysis of A β molecules in brains from adult ovxed female rats treated with E+hCG or E+veh was performed. A β molecules from whole brains ($n=6$ per group) were labeled by 4G8 (panel A), anti-A β 40 (panel B), or anti-A β 42 (panel C). A β molecules from hippocampi samples were detected with anti-rodent A β m3.1 (panel D). Dimers (A, B and C) or monomers (D) of A β molecules were labeled (arrow heads). Each lane in A–C represents one rat brain and typical results are shown. For D, immunoprecipitation was performed with pooled samples (7 females per group). X marks indicate non-specific signals created by immunoprecipitation. Mo: monomers, Di: dimers. Panel E shows densitometry analysis for anti-A β 40 and anti-A β 42 blots. A β 40 and A β 42 dimers were significantly increased in the hCG-treated group compared to the E+veh treated group ($p=0.034$ and $p=0.050$, respectively; $n=6$ per group).

2001), hCG-treated and vehicle-treated groups in the present studies did not differ in activity in an open field or anxiety as measured by the time spent in the center of an open field. Consistent with this, there were no group differences in the amount of object exploration during exposure trials for the object location memory task. Task performance improved in hCG-treated animals when the task was made less demanding through shortened intertrial delay indicating that group differences were not due to inability to do the task. All groups showed robust spatial memory performance for short intertrial delay, treatment-dependent performance during intermediate delay, and at-chance performance with long intertrial delay as would be expected in memory-dependent performance.

Our studies of hCG's disruption of E's enhancement of spatial memory performance in rats are consistent with the small amount of recently published data suggesting that long-term elevation of LH may affect memory performance in mice. Transgenic mice that over-express LH throughout their lives show disruption in hippocampally-associated cognitive performance in adulthood as measured by the Y-maze (Casadesus et al., 2007). Further, LH ablation for 3 mo. in an AD mouse model improved cognitive ability indicated through sustained spontaneous alternation behavior in a Y-maze task (Casadesus et al., 2006). Together with the present experiments, these studies implicate LH in mediating hippocampally-dependent memory function in both mice and rats. The present studies also indicate that relatively short exposure (i.e. hours) to high levels of LH/hCG is effective.

It is not known if LH would counteract estradiol's effect on all the types of learning and memory that estrogen may modulate. E has been shown to have a multitude of effects on a variety of aspects of learning and memory. Generally, exogenous or endogenous estrogens improve performance on spatial memory tasks that involve working memory but estrogens may also have modulatory effects on a broad range of other tasks associated with learning and memory including hippocampus-independent tasks, verbal tasks, conditioning tasks, aversive tasks, and reference as well as working memory tasks (see Dohanich, 2002 for review). Moreover, the effects of estrogen on many of the processes involved in learning and memory in females may vary with the particular task, pattern/amount of hormone exposure, and/or age of the female and it has also been suggested that estrogens may affect the type of cognitive strategy used by an animal (see Daniel, 2006 for review). There are data to suggest that at least some of the effects of estrogen on verbal memory in women may be due to a direct effect of estrogen rather than due to an effect on LH. For example, women with decreased levels of LH and estrogen (due to administration of a gonadotropin releasing hormone agonist) have lower immediate and delayed paragraph recall scores compared to controls, and the decreased verbal memory can be recovered, at least partially, with the addition of estrogen (Sherwin and Tulandi, 1996). The present experiments used a nonaversive, hippocampus-dependent (Mumby et al., 2002) spatial working memory task in adult ovx female rats with estradiol implants. It will be interesting to see how far these effects of LH can be extended.

It is not known if all of the memory-enhancing effects of E on hippocampus-dependent spatial memory are due to estradiol's negative feedback effects on LH or if E has more direct effects of its own. Most studies of estrogen's effects on spatial memory have compared ovxed females with or without E and have ignored the changes in LH that occur with estradiol treatment. In the present studies, supplemental hCG eliminated most of the effect of E on spatial memory. However, it is still possible that both LH and E act at the hippocampus to affect memory. Consistent with this possibility, a few studies have used direct bath application of E to hippocampal slices and reported an increase in long-term potentiation, a cellular model for memory (Bi et al., 2000; Foy et al., 1999). However, it has been suggested that estrogen *in vitro* suppresses LH receptors so the *in vitro* effect of E could still be via suppression of LH signaling (Bowen et al., 2004). Estradiol treatment of ovxed females is also known to increase hippocampal spine density (Gould et al. 1990; Leranth et al. 2000, 2002; Woolley et al. 1990; Woolley and McEwen 1992), a change that is thought to underlie changes in memory ability, but it is unknown if any of the effects of E on spine density may actually be due to changes in LH.

While the mechanism by which hCG mediates spatial memory performance is unknown, the LH/hCG receptor is implicated. The hippocampus, a structure critical to spatial memory, holds the highest concentration of LH/hCG receptors in the CNS (Lei et al., 1993). Transgenic mice over-expressing LH but with silenced receptors did not show cognitive impairment in the Y-maze task suggesting receptor-mediated effect of LH on these behaviors (Casadesus et al., 2007). However, LH can exert some of its effects via mechanisms independent of the LH receptor and traditional downstream signaling mechanisms (West and Cook, 1992; Yarram et al., 2003). Whether or not these receptor-independent effects play a role in this system is yet to be elucidated.

Whether the cognitive impairment of the hCG-treated rats observed in this study is induced by elevated levels of A β remains to be clarified. However, increased A β oligomers after hCG treatment may have contributed to spatial memory deficits in rats as high concentrations of A β 40 and A β 42, particularly A β 42, are considered to be neurotoxic and are correlated with cognitive deficits in AD (Naslund et al., 2000). A β oligomers are reported to disrupt long-term potentiation (LTP) *in vitro* (Wang et al., 2001, 2004) and *in vivo* (Walsh et al., 2002), and, appear to introduce memory impairment in AD mouse models (Cleary et al., 2004). *In vitro* research indicates that LH may increase A β by driving amyloid- β precursor protein (A β PP) processing to the amyloidogenic pathway (Bowen et al., 2004). However, whether LH/hCG affects A β PP processing via LH receptors or through an LH receptor-independent pathway is not known.

LH increases may be responsible for cognitive decline and vulnerability to AD in women previously attributed to loss of E. Post-menopause E loss has been associated with cognitive decline (Birge et al., 2001; Genazzani et al., 1992) and the susceptibility of women to AD, as women have AD 2:1 compared to men (Breitner et al., 1988; Brookmeyer et al., 1998; Jorm et al., 1987; McGonigal et al., 1993; Rocca et al., 1991). However, increases in LH following menopause may be

influential as women experience a 3- to 4-fold increase in the concentration of serum LH following menopause (Chakravarti et al., 1976). LH, rather than E may be responsible for gender-linked patterns in cognitive decline and AD.

LH's role in memory may shed light on problematical findings in hormone replacement therapy (HRT) in women. E's positive effect on memory helped inspire the development of estrogen-based HRT to counteract cognitive senescence following menopause. Although a number of studies have shown that HRT lessens the risk of AD in post-menopausal women, reports from the Women's Health Initiative study indicated that HRT initiated late after menopause (at ages 65 and above) did not improve cognitive performance or lessen AD risk (Shumaker et al., 2003). Although cognitive decline can be rescued with estrogen therapy initiated immediately after ovariectomy, HRT initiated after a longer interval is ineffective (Sherwin, 2005). Success with early treatment of HRT rather than late treatment may be due to the fact that LH is extremely elevated immediately following menopause or ovariectomy and over time the negative feedback system of the hypothalamic-pituitary-gonadal axis becomes unresponsive, so the estrogen in late treatment individuals no longer completely lowers LH. Early HRT treatment may be successful in improving cognition through gonadotropin ablation rather than through estrogen supplementation (Smith et al., 2003). Additionally, there is evidence that after ovariectomy, HRT that consists of conjugated equine estrogens is not effective in counteracting cognitive decline. This may be because conjugated equine estrogens only partially reverse ovariectomy-induced increases in serum gonadotropin levels (Utian, 1978). The previously overlooked role of LH may explain these findings.

In conclusion, inverse levels of LH may be responsible for the effects on spatial memory previously ascribed to estradiol, and LH may have its depressive effects on memory through an increase in amyloid- β in the brain, perhaps the hippocampus. This may have importance for age-related cognitive declines in humans and neurodegenerative diseases like AD. Further research on how LH/hCG exerts its effects and the possible therapeutic value of treatments that modify LH may be important avenues to explore.

Acknowledgments

This work was supported in part by a Research and Development grant from Oberlin College and grants AG10491 and AG05891 from the National Institutes of Health. The authors thank PM Mathews Ph.D. Center for Dementia Research, Nathan Kline Institute, for his kind gift of m3.1 antibody, Jon Levine Ph.D. Northwestern University for his assistance in protocol design, Dennison Smith, Ph.D. Oberlin College for brain dissection, Chaelon Myme Ph.D. Oberlin College for his help with the Barnes maze, and Anne Cherry for her assistance in behavioral testing.

References

Barnes, C.A., 1979. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psych.* 93, 74–104.

- Bi, R., Broutman, G., Foy, M.R., Thompson, R.F., Baudry, M., 2000. The tyrosine kinase and mitogen-activated protein kinase pathways mediate multiple effects of estrogen in hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3602–3607.
- Bimonte, H.A., Denenberg, V.H., 1999. Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology* 24, 161–173.
- Birge, S.J., McEwen, B.S., Wise, P.M., 2001. Effects of estrogen deficiency on brain function. Implications for the treatment of postmenopausal women. *Postgrad. Med. Spec. No.* 11–16.
- Bowen, R.L., Isley, J.P., Atkinson, R.L., 2000. An association of elevated serum gonadotropin concentrations and Alzheimer disease? *J. Neuroendocrinol.* 12, 351–354.
- Bowen, R.L., Smith, M.A., Harris, P.L., Kubat, Z., Martins, R.N., Castellani, R.L., Perry, G., Atwood, C.S., 2002. Elevated luteinizing hormone expression colocalizes with neurons vulnerable to Alzheimer disease pathology. *J. Neurosci. Res.* 70, 514–518.
- Bowen, R.L., Verdile, G., Liu, T., Parlow, A.F., Perry, G., Smith, M.A., Martins, R.N., Atwood, C.S., 2004. Luteinizing hormone, a reproductive regulator that modulates the processing of amyloid- β precursor protein and amyloid- β deposition. *J. Biol. Chem.* 279, 20539–20545.
- Breitner, J.C., Silverman, J.M., Mohs, R.C., Davis, K.L., 1988. Familial aggregation in Alzheimer's disease: comparison of risk among relatives of early and late-onset cases, and among male and female relatives in successive generations. *Neurology* 38, 207–212.
- Brenot, J., Domench, A., Bata, L., 1952. Effect of estrogen administration on leukocyte count in white castrated rats. *C. R. Seances. Soc. Biol. Fil.* 146, 1149–1151.
- Brookmeyer, R., Gray, S., Kawas, C., 1998. Projections of Alzheimer disease in the United States and the public health impact of delaying disease onset. *Am. J. Public Health* 88, 1337–1342.
- Casadesus, G., Webber, K.M., Atwood, C.S., Pappolla, M.A., Perry, G., Bowen, R.L., Smith, M.A., 2006. Luteinizing hormone modulates cognition and amyloid- β deposition in Alzheimer APP transgenic mice. *Biochim. Biophys. Acta* 1762, 447–452.
- Casadesus, G., Milliken, E.L., Webber, K.M., Bowen, R.L., Lei, Z., Rao, C.V., Perry, G., Keri, R.A., Smith, M.A., 2007. Increases in luteinizing hormone are associated with declines in cognitive performance. *Mol. Cell. Endocrinol.* 269, 107–111.
- Chakravarti, S., Collins, W.P., Forecast, J.D., Newton, J.R., Oram, D.H., Studd, J.W., 1976. Hormonal profiles after the menopause. *Br. Med. J.* 2, 784–787.
- Cleary, J.P., Walsh, D.M., Hofmeister, J.J., Shankar, G.M., Kuskowski, M.A., Selkoe, D.J., Ashe, K.H., 2004. Natural oligomers of the amyloid- β protein specifically disrupt cognitive function. *Nat. Neurosci.* 8, 79–84.
- Daniel, J.M., 2006. Effects of oestrogen on cognition: what have we learned from basic research? *J. Neuroendocrinol.* 18, 787–795.
- Dohanich, G., 2002. Gonadal steroids, learning, and memory. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), *Hormones Brain and Behavior*, vol. 2. Academic Press, New York, pp. 256–327.
- Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav. Brain Res.* 31, 47–60.
- Ennaceur, A., Neave, N., Aggleton, J.P., 1997. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Exp. Brain Res.* 113, 509–519.
- Foy, M.R., Xu, J., Xie, X., Brinton, R.D., Thompson, R.F., Berger, T.W., 1999. 17 β -estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J. Neurophysiol.* 81, 925–929.
- Freeman, M.E., Dupke, K.C., Croteau, C.M., 1976. Extinction of the estrogen-induced daily signal for LH release in the rat: a role for the proestrous surge of progesterone. *Endocrinology* 99, 223–229.
- Gallo, R.V., Johnson, J.H., Kalra, S.P., Whitmoyer, D.I., Sawyer, C.H., 1972. Effects of luteinizing hormone on multiple-unit activity in the rat hippocampus. *Neuroendocrinology* 9, 149–157.
- Genazzani, A.R., Gastaldi, M., Bidzinska, B., Mercuri, N., Genazzani, A.D., Nappi, R.E., Serge, A., Petraglia, F., 1992. The brain as a target organ of gonadal steroids. *Psychoneuroendocrinology* 17, 385–390.
- Gibbs, R.B., 1999. Estrogen replacement enhances acquisition of a spatial memory task and reduces deficits associated with hippocampal muscarinic receptor inhibition. *Horm. Behav.* 36, 222–233.

- Gould, E., Woolley, C.S., Frankfurt, M., McEwen, B.S., 1990. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci.* 10, 1286–1291.
- Hogervorst, E., Combrinck, M., Smith, A.D., 2003. Testosterone and gonadotropin levels in men with dementia. *Neuro. Endocrinol. Lett.* 24, 203–208.
- Hogervorst, E., Bandelow, M., Combrinck, M., Smith, S.D., 2004. Low free testosterone is an independent risk factor for Alzheimer's disease. *Exp. Gerontol.* 39, 1633–1639.
- Jones, H.E., Waynforth, H.B., Pope, G.S., 1961. The effect of mesterol on vaginal cornification, pituitary function and pregnancy in the rat. *J. Endocrinol.* 22, 293–302.
- Jorm, A.F., Korten, A.E., Henderson, A.S., 1987. The prevalence of dementia: a quantitative integration of the literature. *Acta Psychiatr. Scand.* 76, 465–479.
- Karsch, F.J., Dierschke, D.J., Weick, R.F., Yamaji, T., Hotchkiss, J., Knobil, E., 1973. Positive and negative feedback control by estrogen of luteinizing hormone secretion in the rhesus monkey. *Endocrinology* 92, 799–804.
- Knowles, F., 1972. Ependyma of the third ventricle in relation to pituitary function. *Prog. Brain Res.* 38, 255–270.
- Koh, J.Y., Yang, L.L., Cotman, C.W., 1990. β -amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. *Brain Res.* 533, 315–320.
- Kuo, Y.M., Emmerling, M.R., Vigo-Pelfrey, C., Kasunic, T.C., Kirkpatrick, J.B., Murdoch, G.H., Ball, M.J., Roher, A.E., 1996. Water-soluble A β (N-40, N-42) oligomers in normal and Alzheimer disease brains. *J. Biol. Chem.* 271, 4077–4081.
- Lambert, J.C., Mann, D., Goumidi, L., Harris, J., Pasquier, F., Frigard, B., Cottel, D., Lendon, C., Iwatsubo, T., Amouyel, P., Chartier-Harlin, M.C., 2000. A FE65 polymorphism associated with risk of developing sporadic late-onset Alzheimer's disease but not with A β loading in brains. *Neurosci. Lett.* 293, 29–32.
- Legan, S.J., Coon, A., Karsch, F.J., 1975. Role of estrogen as initiator of daily LH surges in the ovariectomized rat. *Endocrinology* 96, 50–56.
- Lei, Z.M., Rao, C.V., 2001. Neural actions of luteinizing hormone and human chorionic gonadotropin. *Semin. Reprod. Med.* 19, 103–109.
- Lei, Z.M., Rao, C.V., Komyei, J.L., Licht, P., Hiatt, E.S., 1993. Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. *Endocrinology* 132, 2262–2270.
- Leranth, C., Shanabrough, M., Horvath, T.L., 2000. Hormonal regulation of hippocampal spine synapse density involves subcortical mediation. *Neuroscience* 101, 349–356.
- Leranth, C., Shanabrough, M., Redmond Jr., D.E., 2002. Gonadal hormones are responsible for maintaining the integrity of spinesynapses in the CA1 hippocampal subfield of female nonhuman primates. *J. Comp. Neurol.* 447, 34–42.
- Loosfelt, H., Misrahi, M., Atger, M., Salesse, R., Vu, M.T., Thi, H.L., Jolivet, A., Guiochon-Mantell, A., Sar, S., Jallal, B., Garnier, J., Milgrom, E., 1989. Cloning and sequencing of porcine LH/hCG receptor cDNA: variants lacking transmembrane domain. *Science* 245, 525–528.
- Lukacs, H., 2001. Rat as model for studying behavior effects of hCG. *Semin. Reprod. Med.* 19, 111–118.
- Lukacs, H., Hiatt, E.S., Lei, Z.M., Rao, C.V., 1995. Peripheral and intracerebroventricular administration of human chorionic gonadotropin alters several hippocampus-associated behaviors in cycling female rats. *Horm. Behav.* 29, 42–58.
- Luine, V.N., Richards, S.T., Wu, V.Y., Beck, K.D., 1998. Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Horm. Behav.* 34, 149–162.
- Luine, V.N., Jacome, L.F., Macluskay, N.J., 2003. Rapid enhancement of visual and place memory by estrogens in rats. *Endocrinology* 144, 2836–2844.
- Mann, D.M., 1988. The pathological association between Down syndrome and Alzheimer disease. *Mech. Ageing Dev.* 43, 99–136.
- Mattson, M.P., Cheng, B., Davis, D., Bryant, K., Leiberburg, I., Rydel, R.E., 1992. β -amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J. Neurosci.* 12, 379–389.
- Mattson, M.P., Tomaselli, K., Rydel, R.E., 1993. Calcium-destabilizing and neurodegenerative effects of aggregated β -amyloid peptide are attenuated by basic FGF. *Brain Res.* 621, 35–49.
- McFarland, K.C., Sprengel, R., Phillips, H.S., Kohler, M., Rosembly, N., Nikolic, K., Segaloff, D.L., Seeburg, P.H., 1989. Lutropin-choriogonadotropin receptor; an unusual member of the G-protein-coupled receptor family. *Science* 245, 494–499.
- McGonigal, G., Thomas, B., McQuade, C., Starr, J.M., MacLennan, W.J., Whalley, L.J., 1993. Epidemiology of Alzheimer presenile dementia in Scotland, 1974–88. *BMJ* 306, 380–383.
- McLean, C.A., Cherny, R.A., Fraser, F.W., Fuller, S.J., Smith, M.J., Beyreuther, K., Bush, A.I., Masters, C.L., 1999. Soluble pool of A β amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* 46, 860–866.
- Morgan, D., Diamond, D.M., Gottschall, P.E., Ugen, K.E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., DiCarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M., Arendash, G.W., 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer disease. *Nature* 408, 982–985.
- Mucke, L., Masliah, E., Yu, G.Q., Mallory, M., Rockenstein, E.M., Tatsuno, G., Hu, K., Kholodenko, D., Johnson-Wood, K., McConlogue, L., 2000. High-level neuronal expression of A β 1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J. Neurosci.* 20, 4050–4058.
- Mumby, D.G., Gaskin, S., Glenn, M.J., Schramek, T.E., Lehmann, H., 2002. Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learn. Memory* 9, 49–57.
- Naslund, J., Haroutunian, V., Mohs, R., Davis, K.L., Davies, P., Greengard, P., Buxbaum, J.D., 2000. Correlation between elevated levels of amyloid β -peptide in the brain and cognitive decline. *JAMA* 283, 1571–1577.
- Oliver, C., Holland, A.J., 1986. Down syndrome and Alzheimer disease: a review. *Psychol. Med.* 16, 307–322.
- Oliver, C., Mical, R.S., Porter, J.C., 1977. Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. *Endocrinology* 101, 598–604.
- Packard, M.G., 1998. Posttraining estrogen and memory modulation. *Horm. Behav.* 34, 126–139.
- Rocca, W.A., Hofman, A., Brayne, C., Breteler, M.M., Clarke, M., Copeland, J.R., Dartigues, J.F., Engedal, K., Hagnell, O., Heeren, T.L., et al., 1991. Frequency and distribution of Alzheimer's disease in Europe: a collaborative study of 1980–1990 prevalence findings. The EURODEM-Prevalence Research Group. *Ann. Neurol.* 30, 381–390.
- Sandstrom, N.J., Williams, C.L., 2001. Memory retention is modulated by acute estradiol and progesterone replacement. *Behav. Neurosci.* 115, 384–393.
- Sherwin, B.B., Tulandi, T., 1996. "Add-back" estrogen reverses cognitive deficits induced by a gonadotropin-releasing hormone agonist in women with leiomyomata uteri. *J. Clin. End. & Metab.* 81, 2545–2549.
- Sherwin, B.B., 2005. Surgical menopause, estrogen and cognitive function in women: what do the findings tell us? *Ann. N. Y. Acad. Sci.* 1052, 3–10.
- Short, R.A., Bowen, R.L., O'Brien, P.C., Graff-Radford, N.R., 2001. Elevated gonadotropin levels in patients with Alzheimer disease. *Mayo Clin. Proc.* 76, 906–909.
- Shumaker, S.A., Legault, C., Rapp, S.R., Thal, L., Wallace, R.B., Ochene, J.K., Hendrix, S.L., Jones III, B.N., Assaf, A.R., Jackson, R.D., Kotchen, J.M., Wassertheil-Smoller, S., Wactawaski-Wende, J., 2003. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* 28, 2651–2662.
- Simpkins, J.W., Green, B.S., Gridley, K.E., Singh, M., de Fiebre, N.C., Rajakumar, G., 1997. Role of estrogen replacement therapy in memory enhancement and the prevention of neuronal loss associated with Alzheimer disease. *Am. J. Med.* 103, 19s–25s.
- Smith, M.A., Perry, G., Atwood, C.S., Bowen, R.L., 2003. Estrogen replacement and risk of Alzheimer disease. *JAMA* 289, 1100 author reply 1101–1102.
- Telegdy, G., Rozsahegyi, G., 1971. Effect of gonadotropins on extinction of an avoidance conditioned reflex and exploratory behaviors in the rat. *Acta Physiol. Acad. Sci. Hung.* 40, 209–214.
- Tomidokoro, Y., Lashley, T., Rostagno, A., Neubert, T.A., Bojsen-Moller, M., Braendgaard, H., Plant, G., Holton, J., Frangione, B., Revesz, T., Ghiso, J., 2005. Familial Danish dementia: co-existence of Danish and Alzheimer

- amyloid subunits (A β and A β) in the absence of compact plaques. *J. Biol. Chem.* 280, 36883–36894.
- Utian, W.H., 1978. Effect of postmenopausal estrogen therapy on diastolic blood pressure and bodyweight. *Maturitas* 1, 3–8.
- Walsh, D.M., Hartley, D.M., Kusumoto, Y., Fezoui, Y., Condron, M.M., Lomakin, A., Benedek, G.B., Selkoe, D.J., Teplow, D.B., 1999. Amyloid β -protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J. Biol. Chem.* 274, 25945–25952.
- Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe, D.J., 2002. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 416, 535–539.
- Wang, H.W., Pasternak, J.F., Kuo, H., Ristic, H., Lambert, M.P., Chromy, B., Viola, K.L., Klein, W.L., Stine, W.B., Krafft, G.A., Trommer, B.L., 2001. Soluble oligomers of β amyloid (1–42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. *Brain Res.* 294, 133–140.
- Wang, Q., Walsh, D.M., Rowan, M., Selkoe, D., Anwyl, R., 2004. β -amyloid induced inhibition of LTP induction involves activation of mGluR5 and the Kinases JNK, cdk5 and p38 MAPK. *J. Neurosci.* 24, 3370–3378.
- West, A.P., Cook, B.A., 1992. The LH receptor cytoplasmic tail is required for desensitization of LH action but not cyclic AMP production. *Biochem. Soc. Trans.* 20, 320s.
- Wise, P.M., Ratner, A., 1980. Effect of ovariectomy on plasma LH, FSH, estradiol, and progesterone and medial basal hypothalamic LHRH concentrations old and young rats. *Neuroendocrinology* 30, 15–19.
- Woolley, C.S., McEwen, B.S., 1992. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J. Neurosci.* 12, 2549–2554.
- Woolley, C.S., Gould, E., Frankfurt, M., McEwen, B.S., 1990. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J. Neurosci.* 10, 4035–4039.
- Yankner, B.A., Duffy, L.K., Kirschner, D.A., 1990. Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. *Science* 250, 279–282.
- Yarram, S.J., Perry, M.J., Christopher, T.J., Westby, K., Brown, N.L., Lamminen, T., Rulli, S.B., Zhang, F.P., Huhtaniemi, I., Sandy, J.R., Mansell, J.P., 2003. Luteinizing hormone receptor knockout (LuRKO) mice and transgenic human chorionic gonadotropin (hCG)-overexpressing mice (hCG $\alpha\beta$ +) have bone phenotypes. *Endocrinology* 144, 3555–3564.