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Neurosteroids Affect Spatial/Reference, Working, and Long-Term Memory of Female Rats

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Female rats take longer to acquire a spatial task during behavioral estrus, when GABA-active progesterone and metabolites are elevated. Whether neurosteroids and neuroactive steroids (neuro(active) steroids), which can act at GABA receptor complexes (GBRs), have activational effects on spatial/reference, working, and long-term memory was investigated. In Experiment 1, ovariectomized Long–Evans rats (N = 107) received oil vehicle or one of six neuro(active) steroids, with varying GBR efficacy (greatest to least efficacious: 5α-pregnan-3α-ol-20-one (THP), 5α-pregnan-3α-ol-11,20-dione, 4-pregnen-3,20-dione, 17α-hydroxyprogesterone, 5-pregnen-3β-ol-20-one sulfate, and 5-androstan-3β-ol-17-one sulfate (DHEAS). Following neuro(active) steroid (3.2 or 6.4 mg/kg) or vehicle sc, rats were tested in a Morris water maze, the following week in a Y maze, and then in an open field. Neuro(active) steroid, but not vehicle, animals had decreased distances to the hidden water maze platform. THP (3.2 and 6.4 mg/kg) animals were faster to find this platform than vehicle animals. In the Y maze, 3.2 mg/kg THP increased percentage correct, but 6.4 mg/kg THP increased latencies to the goal box. DHEAS had the opposite effect, with 3.2 mg/kg increasing latencies to the goal box, while 6.4 mg/kg increased percentage correct. In Experiment 2, N = 75 ovariectomized rats were icv implanted with one of the neuro(active) steroids or cholesterol vehicle and then tested for spatial/reference memory, working and long-term memory, and motoricity/anxiolysis as in Experiment 1. DHEAS implants decreased, while THP increased, latencies and distances to the hidden platform in the Morris water maze. In the Y maze, THP increased latencies and decreased percentage correct, but DHEAS increased the likelihood of correct choice. Open field behavior of animals administered the various neuro(active) steroids (sc or icv) was not different. Thus, of the neuro(active) steroids examined, the neurosteroids THP and DHEAS had the most pronounced activational affects on spatial/reference, working, and long-term memory, independent of motoricity.

The sex difference in people's (McGee, 1979; Gaulin & FitzGerald, 1986) and laboratory animals' (Beatty, 1992) performance on spatial tasks, although small, is one of the more reliable individual differences in cognitive ability. This sex difference has been attributed to the organizational effects of sex hormones during the perinatal period. The most convincing are data from laboratory animals which indicate that neonatally androgenized rats show better maze ability than nonandrogenized control females (Roof & Havens, 1992; Binnie-Dawson & Cheung, 1982) and neonatally castrated males (Williams, Barnett, & Meck, 1990). In addition, the spatial ability of people exposed to atypical levels of sex hormones *in utero* indicates that perinatal androgenization may contribute to sex differences in spatial ability (McCormick & Witleson, 1991; Reinisch & Sanders, 1992).

Although steroids' organizational effects on spatial ability are clear, whether sex hormones have activational effects on cognitive function is controversial. In some studies, androgen levels are related to people's spatial performance (Christiansen & Knussman, 1987; Gouchie & Kimura, 1991; McKeever & Deyo, 1990; Shute, Pellegrino, Hubert, & Reynolds, 1983), but other reports failed to see correlations (Hassler, 1992; McKeever, Rich, Deyo, & Conner, 1987). Subtle differences in perceptual-spatial performance have been reported across the men-

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strual cycle (McCormick & Wittleson, 1991; Reinisch & Sanders, 1992; Konenich, Lane, Dickey, & Stone, 1978), during and postpregnancy (Woodfield, 1984; Silverman & Phillips, 1993) and in surgically menopausal women receiving steroid replacement (Sherwin, 1988). The few studies that have investigated the activational effects of sex hormones on spatial ability using animal models have revealed that phase of estrous cycle influences acquisition of a spatial task (Frye, 1995), but once a task is acquired, gonadectomy has a minor influence on spatial performance (Joseph, Hess, & Birecree, 1978; Williams et al., 1990; Frye, 1995).

The majority of research examining hormones' activational effects on females' memory has concentrated on estrogen. It is clear that estrogen may affect memory; however, the direction of estrogen's effects is contingent upon the type of memory assessed. For example, there is a negative correlation between estrogen and spatial ability (Konenich et al., 1978; Silverman & Phillips, 1993; Woodfield, 1984) and a positive correlation between estradiol and paired-associate learning (Phillips & Sherwin, 1992). Although progesterone and its metabolites fluctuate concurrently with estradiol over the estrous/menstrual cycle and pregnancy (Ichikawa, Sawada, Nakamura, & Moroika, 1974; Holzbauer, 1975; Freeman, Purdy, Coutifaris, Rickels, & Paul, 1993), whether progestins affect females' cognitive functioning has not been investigated.

Although it is generally accepted that steroids have their actions by entering cells and interacting with the genome, recent evidence indicates that membrane-limited effects of steroids can produce similar behavioral effects to those of steroids that can act intracellularly (Frye, Mermelstein, & DeBold, 1992b). Biochemical and electrophysiological studies indicate many steroids, such as progesterone (Harrison, Majewska, Harrington, & Barker, 1987; Majewska, Harrison, Schwartz, Barker, & Paul, 1986), estrogen (Hamon, Goetz, Euvarard, Pasqualini, Le Dafneit, Kerdelhue, Cesselin, & Peillon, 1983; Maggi & Perez, 1984; O'Connor, Nock, & McEwen, 1988; Schumacher, Coirini, & McEwen, 1989), their precursors, and their metabolites, can have rapid, nongenomic actions via GABA receptor complexes (GBRs) located on neuronal membranes. Interestingly, steroids are not equally effective at GBRs; in fact, a precursor or a steroid metabolite can have a stereospecific effect at modulating GBRs disparate of its parent compound. For example, *in vitro* progesterone is relatively ineffective on some measures of GBR activity (Belelli, Lan, & Gee, 1990), while the most potent progestins at GBRs are the 5α-reduced metabolites of progesterone, 5α-pregnan-3α-ol-20-one (THP or allopregnanalone), and 5α-pregnan-3α-ol-11,20-dione (THDOC). The former is a neurosteroid because it is produced and has its effects in the brain; the latter is a neuroactive steroid because central synthesis has not been ascertained but THDOC is presumed to have its actions in the CNS. Less effective and inconsistent in their effects at GBRs are the neuroactive steroids 4-pregnen-3,20-dione (P) and 17α-hydroxyprogesterone (17OHP). Finally, 5-pregnen-3β-ol-20-one sulfate (PS) and 5-androstan-3β-ol-17-one sulfate (DHEAS) are neurosteroid antagonists at GBRs (Majewska, Demirgoren, Spivak, & London, 1990; Majewska, 1992). This well-established differential activity of neuro(active) steroids has enabled systematic investigation of the relative importance of GBRs and/or particular neuro(active) steroids in behavioral manifestations (Frye & Duncan, 1994; Frye & Duncan, 1995).

Although the behavioral significance of progestin/GBR interactions is not as well understood as the biochemical relationship, GABA/neuro(active) steroid interaction underlies the rapid anesthetic, anticonvulsant (Belelli et al., 1990), anxiolytic (Bitran, Purdy, & Kellogg, 1993), and analgesic (Frye & Duncan, 1995) action of some neuro(active) steroids. There is evidence that neuro(active) steroids also affect memory. For example, the stimulated release of DHEAS, the precursor to both androgens and estrogens and an allosteric antagonist of GBRs (Majewska et al., 1990), is reduced in both aging and chronically stressed mice and humans (Parker, Levin, & Lifrak, 1985; Parker & Odell, 1980). Administration of DHEA and its sulfate enhances aging male mice's retention for footshock active avoidance and performance in a T-Maze (Flood, Morley, & Roberts, 1992; Flood & Roberts, 1988; Flood, Smith, & Roberts, 1988). Although DHEAS enhancement of long-term memory has been investigated only in males, females also show age-related decrements in memory. In initial stages of Alzheimer's disease, women have greater decrements in naming, verbal fluency, and delayed recall (Henderson & Buckwalter, 1994; Buckwalter, Sobel, Dunn, Diz, & Henderson, 1993). When compared to men, women perform worse on spatial tasks, and among Alzheimer's patients, women's memory decrements are more pronounced. Given these findings, it is surprising that research on steroidal memory enhancement has not employed a female model.

Also surprising is that cyclic fluctuations of hormones' effects on memory in younger animals have received little attention. Decrements in spatial

(Frye, 1995) and long-term memory functioning occur during estrus (Arushanian, Borovkova, Koneev, & Tereshkin, 1988) and pregnancy (Sharp, Brindle, Brown, & Turner, 1993), when there are peak levels of progesterone and its 5α-reduced metabolite, THP (Holzbauer, 1975). In women, increases in progesterone and THP correlate with confusion, fatigue, and delayed verbal recall (Freeman et al., 1993).

Thus, evidence suggests that these two aforementioned neurosteroids, DHEAS and THP, which have opposite effects at GBRs, may be associated with memory changes. In fact, THP is the most potent naturally occurring steroidal agonist of GBRs, while DHEAS is an allosteric antagonist of GBRs. To ascertain whether neuro(active) steroids can activationally modulate memory of younger female animals, the following experiment was conducted.

EXPERIMENT 1

Method

Subjects and Housing

Female Long–Evans rats (N = 107), 55 days of age, were obtained from Charles River Laboratories (Wilmington, MA) and ovariectomized. Animals were individually housed in hanging stainless steel cages (24 × 18 × 19 cm) in a temperature-controlled room (21 ± 1°C) and were maintained on a reverse 12:12-h light:dark cycle (lights on at 2100). All rats had constant access to Purina chow and tap water in their cages. Behavioral testing began 1 h after lights out.

Apparatus

Reference/spatial memory task. A version of the original Morris water tank (200 cm in diameter and 71 cm deep) was utilized as a discriminative assay of spatial ability (Morris, 1981, 1984). The platform was made of wire mesh (5.3 × 5.3 × 33.5 cm) and painted white. There were many constant extramaze visual clues in the room including the experimenter, counter, video camera, and computer equipment. The video camera, which was situated above the water tank, was connected to a computer tracking system (Multitracker, San Diego Instruments) which automatically measured latency and distance of the path each rat took to the platform. The average latency and distance of trials 1–3 and 4–6 were used for statistical analysis.

The tank was filled with 20–22°C water 36 cm deep. The water was colored white by adding a small quantity of powdered milk. The opaqueness of the water enabled the platform to be concealed approximately 2.5 cm below the surface of the water and 30 cm from the side of the tank.

Procedure

Rats were taken in their home cages to the room containing the water maze. Each rat was tested for six trials on each of 2 consecutive days. On the first day of testing, all rats received 0.2 cc SC of sesame oil vehicle; on Test Day 2, either sesame oil vehicle (N = 8) or 3.2 (N = 51) or 6.4 mg/kg (N = 48) of one of six neuro(active) steroids (DHEAS, PS, 17OHP, P, THDOC, or THP), suspended in 0.2 cc sesame oil vehicle, was administered. This schedule ensured all rats had some familiarity with the task and avoided possible memory distortion effects of post-trial hormonal treatment (Carey, 1987; Bitran et al., 1993; Frye & Duncan, 1994).

All injections occurred 30 min prior to the first trial. Each trial was initiated at one of three starting positions, located 43, 198, or 253° clockwise from the platform's location, as was assigned randomly by the tracking program. Rats were placed in the water at the designated starting location and the experimenter initiated the tracking system. If a rat failed to reach the platform within 120 s, it was guided to the platform by the experimenter. The rat remained on the platform for 45 s to orient itself to visual cues. After completing a trial, rats were returned to their cage for a 3-min intertrial period, until six trials were completed. Because the platform in the water maze remained in the same location for each trial, performance on this spatial task was used to assess neuro(active) steroids effects on reference memory.

Working/long term memory task. To confirm that the neuro(active) steroids' effects upon spatial/reference memory were not solely a function of altering performance, working and long-term memory were also examined (McGaugh, 1989a,b). Delayed nonmatching to sample (DNMTS) was used as an assay of working and long-term memory (Kelsey & Vargas, 1993). Training occurred in a Y maze, which consisted of a start arm (61 cm long, 13 cm wide, and 30 cm high) and two goal boxes (46 × 15 × 30 cm). A guillotine door enclosed the start box (23 cm length), which led to a triangular choice area (15 × 15 × 15 cm) and to either of the goal arms. Enclosing each goal box was a guillotine door, similar to the start door, located 30 cm from the end of the goal arm. Positioned 0.5 cm from the end of each goal arm was a metal cup (1 cm in diameter). To ensure

sound stimuli were constant during both habituation and testing, a white noise generator was used.

Procedure

Habituation, which took 2 days to acquire, began 1 week after water maze testing. Rats were water deprived for 72 h prior to habituation and sc injected with 0.2 cc of sesame oil vehicle on both habituation days.

On Habituation Day 1, rats explored the maze without the doors blocking the arms of the maze and drank 0.5 cc of 8% sugar water from the metal cups located in each of the goal arms. Rats were then placed in the Y maze start box with the door down. After 5 s the door was lifted and closed when the rat had traveled past it. Then the rat again explored the maze and drank from both cups of 8% sugar water. This procedure enabled rats to become accustomed to, and drink in, the novel environment.

On Habituation Day 2, rats were placed in the start box with the door down. After 5 s, the door was lifted and lowered after the rats had traversed past. One goal box was open and baited with sugar water (forced arm), while the other remained closed. After the rat had passed through the forced arm, the experimenter shut the door, and the rat drank the sugar water. Rats were only given sugar water in one goal box at a time, so that in the next part of the procedure (testing), their performance in a free choice situation (both goal boxes open) could be assessed. Habituation was completed once the rat had successfully run three consecutive forced trials in under 120 s each. Testing began the next day and ran for 2 days (Day 3 and Day 5).

The 56 rats previously receiving 6.4 mg/kg neuro(active) steroid ($N = 48$) or vehicle ($N = 8$) were injected with the same neuro(active) steroid or vehicle they had received prior, but on Day 3 they received 3.2 mg/kg and on Day 5 6.4 mg/kg. The rats tested in the water maze following 3.2 mg/kg were not used in this experiment.

Testing consisted of 10 trials of a forced run immediately followed by a choice run. For the choice run, both goal arm doors were opened, and the rat was allowed to choose either arm. If the rat chose the arm it had just visited in the forced run (incorrect choice), the door would be shut and the rat remained in the box for 25 s with no sugar water. If the rat chose the opposite arm than it was forced down in the previous run (correct choice), the rat had sugar water and was removed from the box after consuming the water. After a rat had completed 1 forced and choice trial, it was placed back into the cage for a 3-min intertrial period. The forced arm run for each trial was randomly chosen so each arm was sampled five times and no arm was chosen three times successively. The latency to the goal arm and the percentage of correct baited arm entries in the choice run were calculated for the 10 trials on Day 3 and Day 5 of testing. The mean latency and percentage of correct of trials 1–5 and 6–10 were used for data analysis. Because rats were expected to recall that both arms were baited (Habituation Day 1) and that a correct choice required alternating between baited arms (Habituation Day 2), this task was considered a measure of long-term memory; because correct choice was trial-dependent, it also assayed working memory.

Open field task. Locomotor activity was measured in an open field divided into four equal chambers (23 × 23 × 15 in each). Positioned above the open field was a video camera that tracked the rats by using the Multitracker system (San Diego Instruments).

Procedure

Rats were injected (0.2 cc) with the same neuro(active) steroid (6.4 mg/kg) assigned during the previous two behavioral tests. Each injected rat was placed in a section of the open field for 20 min. Rats were tracked for four 5-min intervals; the distance each rat traveled was calculated by the program.

General Procedure

Rats were tested for 2 consecutive weeks; during the first week rats were tested for spatial/reference memory in the Morris water maze. The following week, working/long-term memory was assessed by Y maze performance. To ascertain whether performance differences were attributable to motor or anxiolytic effects, open field behavior was examined.

Results

Spatial/Reference Memory Task

Latency to platform. Three-way repeated measures ANOVA revealed that subcutaneous administration of 3.2 and 6.4 mg/kg produced main effects of NEURO(ACTIVE) STEROID $F(6, 223) = 3.24$, $p \leq .05$; $F(6, 203) = 4.34$, $p \leq .05$; DAY OF TESTING $F(1, 223) = 118.45$, $p \leq .001$; $F(1, 203) = 125.4$, $p \leq .001$; and TRIAL $F(1, 223) = 66.29$, $p \leq .001$; $F(1, 203) = 116.01$, $p \leq .001$. Interactions were different for the two sc dosages.

For those receiving 6.4 mg/kg, there was an interaction between DAY OF TESTING and TRIALS $F(1,$

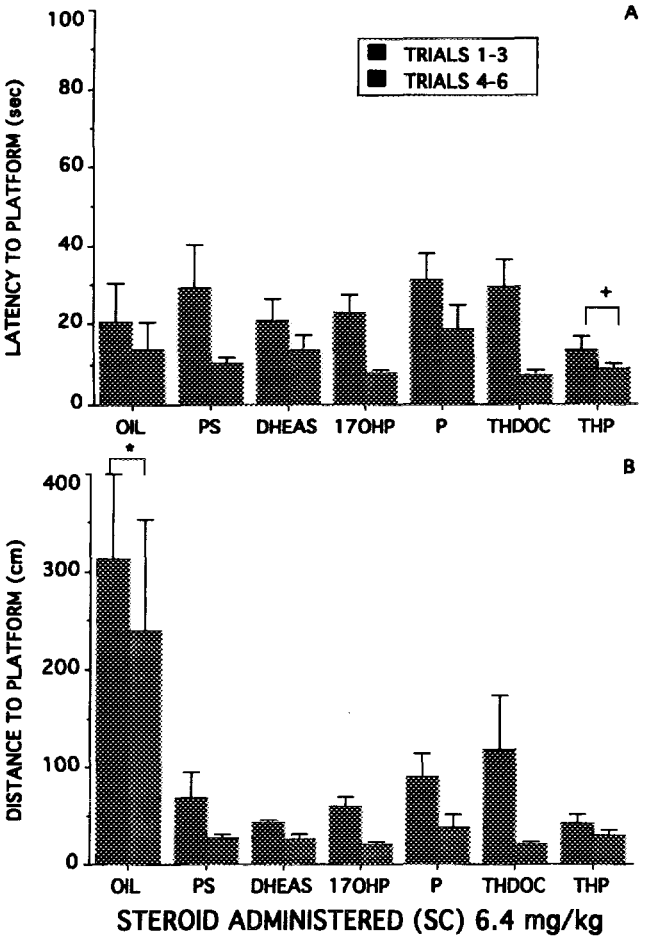

FIG. 1. (A) Mean latencies ± standard error of the mean for rats to reach the hidden platform in the Morris water maze when administered 6.4 mg/kg of neuro(active) steroid; (+) indicates that rats receiving THP had significantly ($p < .05$) lower latencies to the platform than did vehicle-administered animals. (B) Mean distances ± standard error of the mean for rats to reach the hidden platform subsequent to sc 6.4 mg/kg of neuro(active) steroid; (*) indicates that rats administered vehicle had significantly ($p < .05$) longer distances to the platform compared to all other animals.

223) = 16.84, $p \leq .001$. Further examination revealed that on Day 1, when all rats received vehicle injections, animals did equally poorly, with some improvement on trials 4–6 (data not shown). Day 2 performance of rats receiving 6.4 mg/kg neuro(active) steroid was significantly enhanced compared to Day 1. When Day 2 performance of all rats receiving 6.4 mg/kg neuro(active) steroid was compared to vehicle controls, only THP-treated animals took a significantly shorter time to find the platform (see Fig. 1A).

For those rats receiving 3.2 mg/kg neuro(active) steroid, there were interactions between DAY OF TESTING and TRIALS $F(1, 203) = 37.54, p \leq .001$; NEURO(ACTIVE) STEROID and DAY OF TESTING $F(6, 203) = 5.19, p \leq .001$; as well as NEURO(ACTIVE) STEROID and TRIALS $F(6, 203) = 2.80$, $p \leq .05$. Again on Day 1, when subjects received oil injections, all animals did poorly but with some improvement on trials 4–6 (data not shown). Day 2 performance of rats receiving 3.2 mg/kg SC neuro(active) steroid was significantly enhanced compared to Day 1, irrespective of steroidal treatment. Consistent with performance of animals receiving 6.4 mg/kg, when Day 2 performance of all rats receiving 3.2 mg/kg neuro(active) steroid were compared to vehicle controls, only THP-treated animals took a significantly shorter time to find the platform (see Fig. 2A).

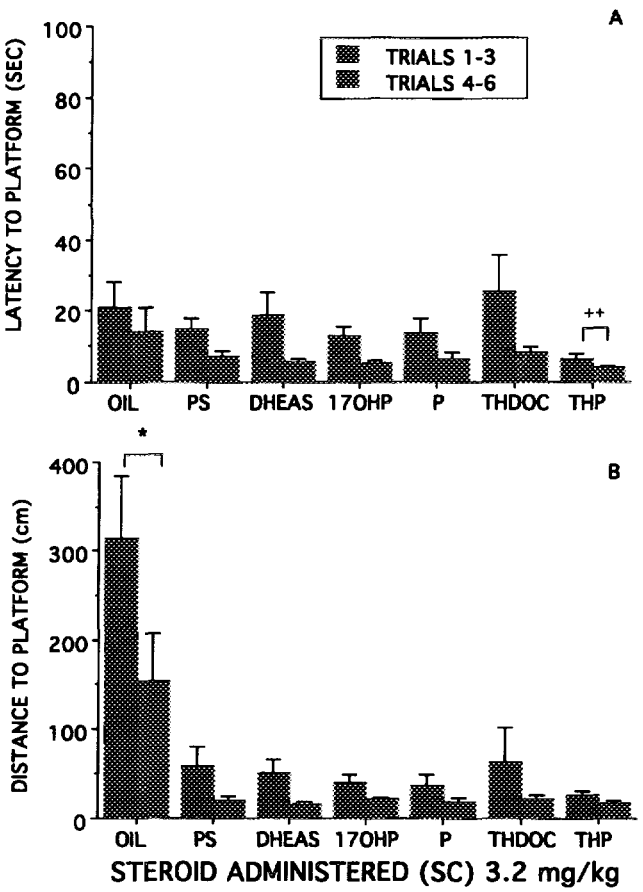

FIG. 2. (A) Mean latencies ± standard error of the mean for rats to reach the hidden platform in the Morris water maze, when administered sc 3.2 mg/kg of neuro(active) steroid; (++) indicates that rats receiving THP had marginally ($p = .08$) lower latencies to the platform than did vehicle-administered animals. (B) Mean distances ± standard error of the mean for rats to reach the hidden platform subsequent to 3.2 mg/kg of neuro(active) steroid; (*) indicates that rats administered vehicle had significantly ($p < .05$) longer distances to the platform compared to all other animals.

Distance to platform. Consistent with measures for latency, for 6.4 and 3.2 mg/kg animals, respectively, there were main effects of NEURO(ACTIVE) STEROID $F(6, 223) = 6.17$, $p \leq .001$; $F(6, 203) = 5.80$, $p \leq .001$ and TRIAL $F(1, 223) = 11.75$, $p \leq .001$; $F(1, 203) = 20.70$, $p \leq .001$ on distance to the hidden platform. For the animals receiving 6.4 mg/kg there was also an interaction between NEURO(ACTIVE) STEROID and DAY OF TESTING $F(1, 223) = 2.33$, $p \leq .05$. On Day 1, all groups took an equally long distance to find the hidden platform; distances were reduced overall on trials 4–6 compared to 1–3 (data not shown). Day 2 performance was improved for all animals, but particularly for animals receiving neuro(active) steroids (3.2 or 6.4 mg/kg) compared to vehicle. No significant differences were noted between neuro(active) steroids. On Day 2, all animals did better on trials 4–6 than trials 1–3 (see Figs. 1B and 2B).

Working/Long-Term Memory Task

Latency to goal box. There were no main effects of NEURO(ACTIVE) STEROID $F(6, 223) = 1.62$, $p \leq .15$ or TRIAL $F(1, 223) = 0.24$, $p \leq .63$, but there was a main effect for DAY OF TESTING $F(1, 223) = 10.35$, $p \leq .003$ and an interaction between DAY OF TESTING and NEURO(ACTIVE) STEROID $F(6, 223) = 6.28$, $p \leq .001$. The interaction is attributable to neuro(active) steroids having different effects depending upon day of testing.

On Day 3, 3.2 mg/kg DHEAS animals showed decrements in working memory. On Day 3, DHEAS animals had a significantly longer latency to the goal box compared to all other animals. Rats receiving P showed impaired performance; 3.2 mg/kg P animals had a longer latency to the goal box than 17-OHP or THP animals. PS rats also had a longer latency to the goal box than THP-treated rats (see Fig. 3A).

On Day 3, 3.2 mg/kg THP rats showed optimal performance, yet on Day 5, when administered 6.4 mg/kg, THP rats did worse. On Day 5, THP rats had significantly longer latencies to the goal box than all neuro(active) steroid receiving animals, save those administered P and vehicle (see Fig. 4A).

Percentage correct. There were no main effects of NEURO(ACTIVE) STEROIDS $F(6, 223) = 1.06$, $p \leq .39$ or TRIAL $F(1, 223) = 0.08$, $p \leq .779$, but there was a main effect for DAY OF TESTING $F(1, 223) = 16.93$, $p \leq .003$ and an interaction between DAY OF TESTING and NEURO(ACTIVE) STEROID $F(6, 223) = 5.03$, $p \leq .001$. The interaction again was attributable to the neuro(active) steroids having different effects on days of testing.

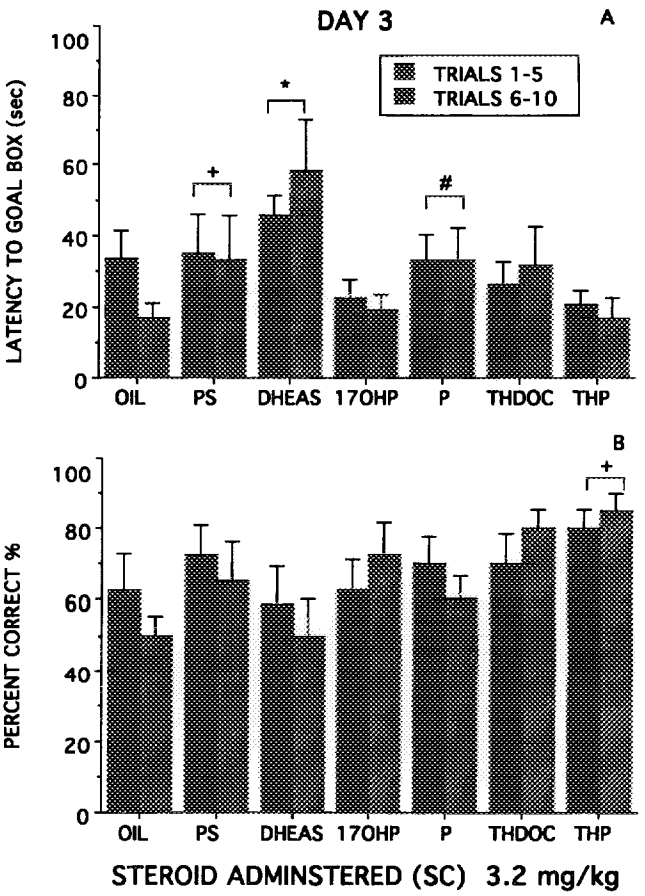

FIG. 3. (A) Mean latencies ± standard error of the mean for rats to reach the Y maze's goal box on Day 3 when administered sc 3.2 mg/kg of neuro(active) steroid; (*) indicates that rats receiving DHEAS had significantly ($p < .05$) longer latencies to the goal box than did all other animals; (+) indicates that PS animals had significantly longer latencies than THP-treated animals; (#) indicates that P-treated animals had longer latencies than did 17OHP or THP animals. (B) Mean percent correct ± standard error of the mean for rats to correctly reach the goal box subsequent to 3.2 mg/kg of neuro(active) steroid; (+) indicates that rats administered THP had significantly ($p < .05$) higher percentage correct compared to oil-, DHEAS-, P-, and THDOC-administered animals.

On Day 3, 3.2 mg/kg THP-treated animals had significantly greater percentage correct than oil-, DHEAS-, P-, or THDOC-treated animals (see Fig. 3B). On Day 5, 6.4 mg/kg DHEAS-treated animals had greater percentage correct than did oil-, PS-, P-, THDOC-, or THP-treated animals (see Fig. 4B).

Open Field Task

There was no main effect of NEURO(ACTIVE) STEROID $F(6, 147) = 1.20$, $p = .32$, but there was a main effect of TIME spent in the open field $F(3, 147) = 7.20$, $p \leq .001$ on distance traveled. Rats'

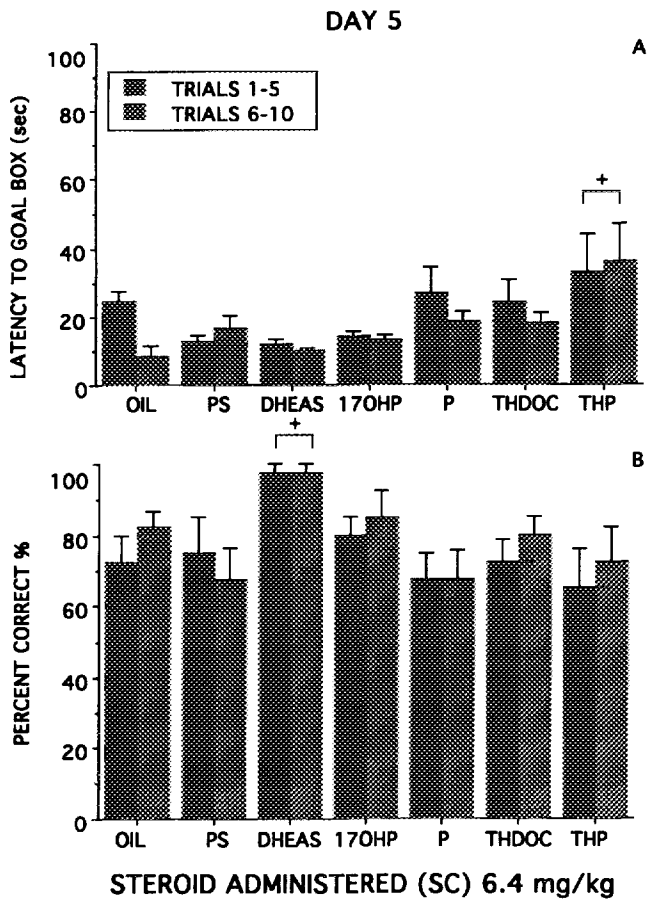

FIG. 4. (A) Mean latencies ± standard error of the mean for rats to reach the goal box in the Y maze following 6.4 mg/kg sc administration of assigned progestin; (+) indicates that THP-treated animals had significantly longer latencies than PS-, DHEAS-, 17OHP-, and THDOC-treated rats. (B) Mean percent correct choices ± standard error of the mean; (+) indicates that rats administered DHEAS had significantly ($p < .05$) greater percentage correct than oil-, PS-, P-, THDOC-, or THP-treated rats.

activity decreased over time regardless of neuro(active) steroid or vehicle (data not shown).

Discussion

The neuroactive steroids examined enhanced spatial/reference, working, and long-term memory. In particular, the neurosteroid most effective at modulating GBRs, THP, enhanced spatial/reference ability most. This is illustrated by THP decreasing latency and distance to the hidden platform following both sc 3.2 and 6.4 mg/kg administration. Allopregnanalone had enhancing effects on working and long-term memory, although limited to the lower dose (3.2 mg/kg). At the higher 6.4 mg/kg dosage, THP rats took significantly longer to reach the goal box. Thus, THP improved spatial ability or reference memory at both dosages tested (3.2 and 6.4 mg/kg), but only enhanced working and long-term memory following 3.2 mg/kg dose.

Interestingly, DHEAS, an allosteric antagonist of GBRs, had no specific effect on spatial ability, but altered working and long-term memory, contingent upon dose administered. Following 3.2 mg/kg DHEAS, significant decrements in working memory were noted, while at 6.4 mg/kg DHEAS, working memory was enhanced. On Day 3, 3.2 mg/kg DHEAS increased latencies to the goal box, but on Day 5, 6.4 mg/kg DHEAS increased percentage correct. Taken together, these results suggest (1) THP produces more salient enhancement of reference than working or long-term memory and (2) DHEAS' memory enhancing effects may be specific to the higher dose and working/long-term memory. Finally, DHEAS' and THP's effects were specific to memory as they did not alter open field behavior.

Due to concerns regarding the possibility that THP and DHEAS's differential solubility affected their penetration of the blood–brain barrier, neuro(active) steroids were also administered centrally. Because dosages seemed to produce different effects in Experiment 1, intraventricular implants were used to ascertain whether the neuro(active) steroids were having effects on memory via CNS actions, irrespective of dosage.

EXPERIMENT 2

Method

With some modifications, the same behavioral tests and procedures were utilized as described for Experiment 1. Seventy-five additional rats were ovariectomized and stereotaxically implanted with chronic cannula in the lateral ventricle as previously described (Frye & Duncan, 1994). Blank inserts were cleaned daily to avoid the cannula becoming occluded and to prevent stress effects during testing. Animals were tested subsequent to implantation for possible neurological deficits produced by the implant, stereotaxic surgery, or neuro(active) steroid (Frye & DeBold, 1993; Frye & Duncan, 1994). Instead of receiving sesame oil for vehicle, inserts were tamped in cholesterol. Test implants were tamped in the neuro(active) steroid as previously described and contained 1–2 μg of neuro(active) steroid (Frye & Duncan, 1994).

One week after surgery, animals had cholesterol or neuro(active) steroid containing inserts placed into their guide cannula. Testing in the water maze

followed immediately. The patency of the cannula was a concern; therefore, rats were tested for 1 day in the water maze. The following week, rats received neuro(active) steroid or cholesterol implants and were tested in the Y maze. Nineteen of 75 animals' cannula had become dysfunctional and the rats were not tested in the Y maze. After the Y maze, open field behavior also was assessed. Rats then were euthanized by decapitation and implant sites in lateral ventricle were verified.

Results

Spatial/Reference Memory Task

Latency to platform. A two-way ANOVA revealed main effects of TRIAL $F(1, 68) = 39.5, p \leq .001$, as well as a trend for a main effect of NEURO(ACTIVE) STEROID $F(6, 68) = 2.05, p \leq .07$. There was also an interaction between NEURO(ACTIVE) STEROID and TRIALS $F(6, 68) = 1.87, p \leq .09$. These effects were attributable to GBR antagonists PS and DHEAS producing significantly shorter latencies compared to controls on trials 1–3. On trials 4–6, THP-treated animals took a significantly longer time to find the platform compared to P-treated animals (see Fig. 5A).

Distance to platform. Consistent with measures for latencies, there were main effects of NEURO(ACTIVE) STEROID $F(6, 68) = 2.63, p \leq .05$, TRIALS $F(1, 68) = 32.45, p \leq .01$ and an interaction between these variables $F(6, 68) = 3.00, p \leq .01$ on distance to the hidden platform. These main effects were due to the shorter distances of animals receiving GBR antagonists PS and DHEAS on trials 1–3 and the longer distances of THP-treated animals on trials 4–6 (see Fig. 5B).

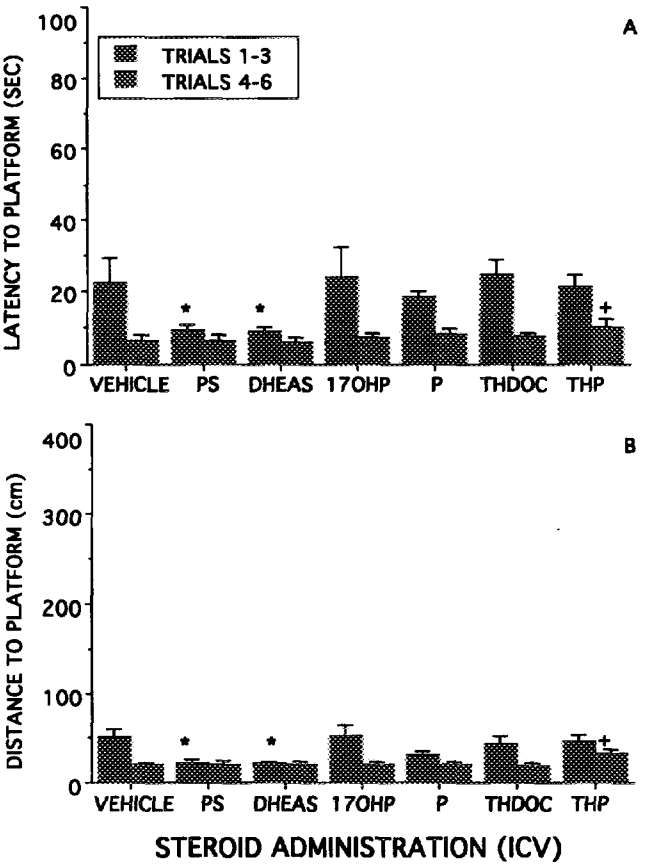

FIG. 5. (A) Mean latencies ± standard error of the mean for rats to reach the hidden platform in the water maze when administered icv neuro(active) steroid or cholesterol implants. (B) Mean distances ± standard error of the mean for rats to reach the hidden platform subsequent to implantation; (*) indicates that rats administered indicated progestin had significantly ($p < .05$) reduced latencies and distances to the platform on trials 1–3 compared to all other groups; (+) indicates that on trials 4–6 THP animals differed significantly from P-treated animals.

Working/Long-Term Memory Task

Latency to goal box. There was a main effect of NEURO(ACTIVE) STEROID $F(6, 49) = 2.95, p \leq .01$, but no effect of trial on latency to the goal box on Day 3. THP rats had longer latencies to the goal box than DHEAS- or THDOC-treated rats (Fig. 6A). On Day 5, the main effect of NEURO(ACTIVE) STEROID $F(6, 49) = 2.89, p \leq .01$) was attributable to THP. THP rats had longer latencies to the goal box than all other animals (Fig. 7A).

Percentage correct. There was a main effect of NEURO(ACTIVE) STEROID $F(6, 49) = 2.53, p \leq .05$, but no effect of trial on percent correct choices on Day 3. DHEAS rats had a significantly greater number of correct choices compared to vehicle animals on Day 3 (Fig. 6B). On Day 5, the main effect of NEURO(ACTIVE) STEROID $F(6, 49) = 3.39, p \leq .01$ was attributable to THP animals being less likely to correctly choose the baited arm compared to all other animals (Fig. 7B).

Open Field Task

There was no main effect of NEURO(ACTIVE) STEROID, but there was a main effect of TIME spent in the open field $F(3, 147) = 23.85, p \leq .0001$. As in Experiment 1, rats were less active over the 20-min period (data not shown).

GENERAL DISCUSSION

These findings suggest that neurosteroids can have activational effects, producing enhancement of

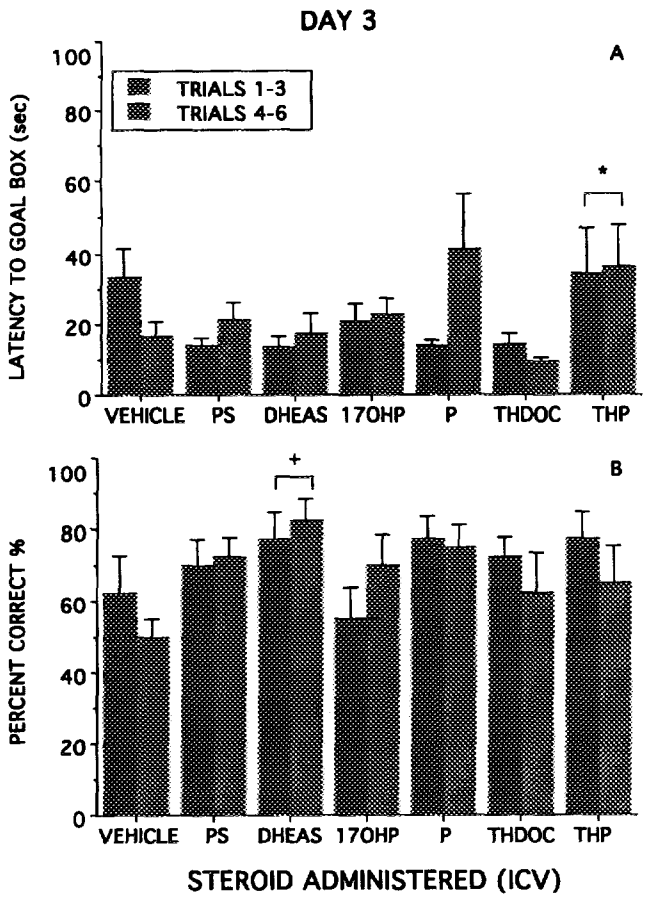

FIG. 6. (A) Mean latencies ± standard error of the mean for rats to reach the Y maze's goal box in choice trials on Day 3 following icv implantation; (*) indicates that rats administered THP had significantly ($p < .05$) longer latencies compared to all other groups. (B) Mean percentage of correct choices to the baited arm in choice trial ± standard error of the mean subsequent to implantation; (+) indicates that rats administered DHEAS had significantly ($p < .05$) higher percentage correct choices when compared to vehicle-administered controls.

spatial/reference, working, and long-term memory, in female rats. The most potent neuro(active) steroid modulators were the neurosteroids THP and DHEAS; THP is a neurosteroid because it is normally produced in the brain (Jung-Testas, Hu, Baulieu, & Robel, 1989), while DHEAS is considered a neurosteroid even though its synthesis hasn't been demonstrated centrally, because CNS concentrations of DHEAS exceed that in the blood and are independent of gonadal and adrenal synthesis (Baulieu, 1991). Both THP and DHEAS have extreme effects at modulating GBRs (Majewska et al., 1986, 1990). The memory effects produced by these neurosteroids were contingent upon type of memory assessed, route of administration, dosage, and neurosteroid administered. Specifically, THP enhanced spatial/reference memory following sc 3.2 and 6.4 mg/kg administration, while working memory was improved only following 3.2 mg/kg THP. Conversely, icv THP produced deficits in both water and Y maze performance. DHEAS enhanced working/long-term memory following 6.4 mg/kg sc administration, whereas spatial, reference, working, and long-term memory were enhanced subsequent to icv administration. Neurosteroid administration did not result in significant differences in open field behavior, which suggest the present activational effects upon memory were independent of motoricity or anxiolysis.

These data confirm and extend the findings of previous researchers in a number of ways. First, we confirmed that DHEAS can enhance long-term mem-

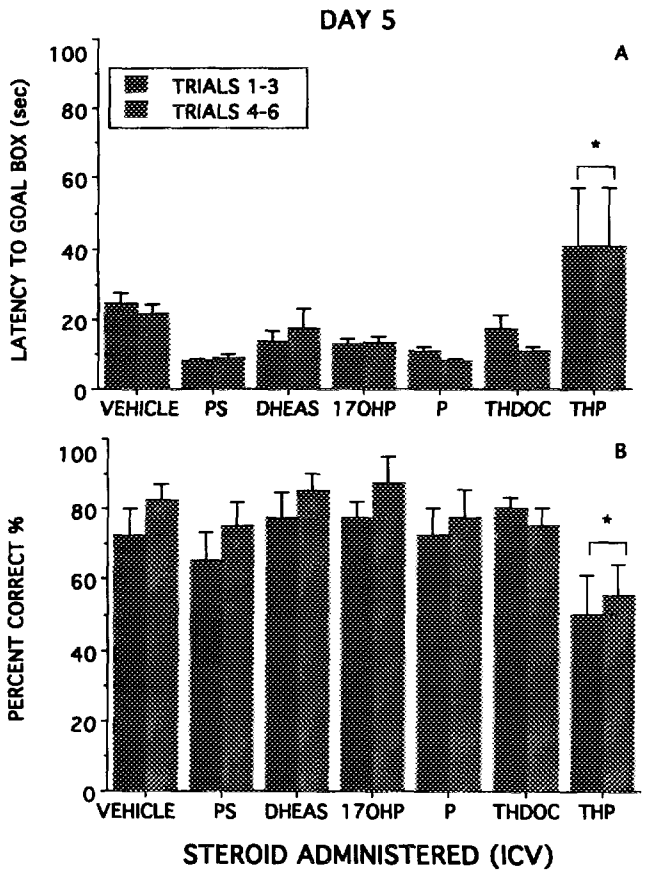

FIG. 7. (A) Mean latencies ± standard error of the mean for rats to reach the Y maze's goal box in choice trials on Day 5 following icv implantation; (*) indicates that rats administered THP had significantly ($p < .05$) longer latencies compared to all other groups. (B) Mean percentage of correct choices to the baited arm in choice trial ± standard error of the mean subsequent to implantation; (*) indicates that rats administered THP had significantly ($p < .05$) reduced percentage correct choices when compared to all other groups.

ory in females, as had been previously ascertained in males; these data also indicate that DHEAS' memory enhancing effects extend to spatial ability (Flood et al., 1992; Flood & Roberts, 1988). Second, pregnanolone, the precursor to THP, and DHEAS produce improvement of mice's retention for footshock avoidance training in a Y maze (Flood et al., 1992). Because THP produced memory enhancement presently, it confirms the hypothesis that pregnenolone's memory enhancing effects were likely due to this neurosteroid serving as a precursor to another, such as THP (Flood et al., 1992). Third, the dosages of DHEAS maximally effective for memory retention in male mice has been reported previously as 162 ng icv and 700 μg sc (Flood et al., 1992; Flood & Roberts, 1988). In the present experiment, 1- to 2-μg implants were effective, as was administration of approximately 800 μg (3.2 mg/kg) or 1600 μg (6.4 mg/kg) sc DHEAS to ovariectomized female rats. This confirms that DHEAS' memory enhancing effects, previously reported by Flood, are not unique to this species, sex, or memory task (Flood et al., 1992, 1988; Flood & Roberts, 1988).

In addition to the present findings confirming previous research on hormonal modulation of aging and memory, these data suggest a GABAergic substrate may underlie cyclic fluctuations in memory. For example, high levels of THP were associated with memory decrements, which is consistent with reported estrous-associated deficits in spatial (Frye, 1995) and long-term memory (Arushanian et al., 1988) which occur when cyclic THP would be relatively higher. Likewise, consistent low THP levels similar to those during diestrus (Holzbauer, 1975; Ichikawa et al., 1974) produced memory enhancement. Females performed best on tasks of spatial ability (Frye, 1995) and long-term memory (Arushanian et al., 1988) during diestrus.

These data confirm our earlier findings (Frye, 1995) that suggest that adrenal and gonadal steroids can have activational effects on memory. What remains to be seen is the role of endogenous THP and DHEAS on reproductive fluctuations in memory. Research has focused on DHEAS because this neurosteroid is reduced in aging and administration to aging mice improves footshock active avoidance training and facilitates retention for step-down passive avoidance training (Flood et al., 1988). Of particular interest to our research is that DHEAS is reduced during prolonged acute stress (Flood et al., 1992), while THP is increased in response to acute stress (Purdy, Morrow, Moore, & Paul, 1991). Interestingly, fluctuations of neurosteroids, such as THP, that are altered in response to environmental stressors contribute to cyclic fluctuations in sexual receptivity (Erskine, 1983; Frye & Debold, 1993, Frye & Leadbetter, 1994; DeBold & Frye, 1994) and analgesia (Frye, Bock, & Kanarek, 1992a; Frye, Cuevas, & Kanarek, 1993; Frye & Duncan, 1994). Thus, as cyclic fluctuations of neurosteroids contribute to changes in sexual behavior and nociception, it follows that endogenous fluctuations in neurosteroids may also effect other behavioral processes, like spatial and long-term memory, that are known to vary concomitant with hormonal and reproductive states, as well as the life span.

These neurosteroids may enhance memory by effecting GBRs, just as $GABA_A$ agonists enhance memory (Jerusalinsky, Quillfeldt, Walz, Da, Bueno, Bianchin, Schmitz, Zanatta, Ruschel, Paczko, Medina, & Izquierdo, 1994; Introini-Collison, Castellano, & McGaugh, 1994). However, GABAergic activation underlies neuro(active)steroids' and other drugs' analgesic, anxiolytic, and anesthetic effects, suggesting that neuro(active) steroids' effects on memory performance may be nonspecific. For example, cyclic fluctuations in pain sensitivity (Frye et al., 1992a,b) are attributable to neuro(active) steroid/GABA interactions (Frye & Duncan, 1994; Frye, van Keuran, & Erskine, 1994). Both THP and DHEAS produce anxiolysis/sedation. Allopregnanolone (1.25–10 μg icv) increases entry and time spent in open arms of an elevated plus maze (Bitran et al., 1993) and (1.25–5 μg icv) decreases rat pups ultrasonic vocalization in response to maternal separation (Zimmerberg, Brunelli, & Hofer, 1994). At the higher aforementioned dosages, THP produces motor incoordination, ataxia, and sedation (Zimmerberg et al., 1994; Bitran et al., 1993); THP and other progestins are also associated with muscle relaxation (Cabral, Gutierrez, Fernandez, Cantabrana, & Hidalgo, 1994). DHEAS enhances the hypnotic and hypothermic effects of ethanol and pentobarbital (Melchior & Ritzmann, 1992). Although it is possible that THP's and DHEAS' present memory enhancing effects were due to these neurosteroids producing these effects, this is unlikely given that dosages lower than those known to produce anxiolysis were used and open field behavior, itself a measure of anxiolysis, was unaffected. Additional support for these neurosteroids' effects being memory specific, and not secondary to ataxia or anxiolysis, include the fact that there are reports of drugs which produce anxiolysis and act at GBRs having effects independent of memory (e.g., benzodiazepines; McNamara & Skelton, 1993).

Alternatively, the neuro(active) steroids may not produce anxiolysis, but they may alter how a task is

perceived in terms of its stressfulness and thereby alter performance (Izquierdo, Pereira, & Medina, 1990; Carey, 1987). For example, DHEAS and THP produce the most extreme nociceptive effects when administered sc or icv to female rats; DHEAS makes animals more sensitive, while THP makes animals less sensitive to painful stimulus (Frye & Duncan, 1994). Further, corticosterone antagonists disruption of consolidation of spatial information (Oitzel & de Kloet, 1992) and flumazenil's memory enhancing effects may be due to these drugs altering the stressful nature of a task (Ferre, Fernandez-Teruel, Escorihuela, Garcia, Zapata, & Tobena, 1994). Alteration of stress/anxiety perception may affect memory by changing the range of stress necessary for optimal performance.

These findings are particularly interesting in light of steroids' ability to change neuronal morphology and exert neuroprotective effects. Increases in dendritic hippocampal spine density correlate well with estrous-associated deficits in spatial ability (Wooley & McEwen, 1993; Frye, 1995). Are neurosteroids such as DHEAS and THP involved in this process? Steroids can alter programmed cell death (Billig, Furuta, & Hsuesh, 1993) and, in particular, testosterone prevents dentate gyrus granular cell layer loss that occurs over development in untreated female rats (Harrell, Goyal, Parsons, & Peagler, 1990; Roof, 1993). Similarly, THP is as effective as MK-801 or nimodipine in preventing deficits in spatial ability and dentate gyrus granular cell layer loss secondary to stimulation of the perforant pathway (Sanderson, Frye, & Kelsey, 1993; Frye, 1995).

Although it is not exactly clear how steroids exert these neuroprotective effects there are several possible mechanisms. Steroids may protect cells by altering NMDA receptors. Evidence for this includes the finding that steroids alter NMDA receptor binding in the hypothalamus and cerebral cortex (Brann, Zamorano, Chorich, & Mahesh, 1993); NMDA receptor-mediated synaptic transmission in the amygdala (Gean, Chang, Huang, Lin, & Way, 1993); and, long-term potentiation in the limbic system (Dubrovsky, Gijsbers, Filipini, & Birmingham, 1993). Steroids may also have their protective effects on neurons by acting via GBRs. Steroids, especially neurosteroids, alter GABA and glutamate release from identified neural terminals in the rat hippocampus (Tauboll, Ottersen, & Gjerstad, 1993) and modulate amino acid neurotransmitter receptors (Farb, Gibbs, Wu, Gyenes, Friedman, & Russek, 1992) and glycine gated ion channels (Farb et al., 1992). Irrespective of the mechanism of steroids' neuroprotective effects, because neuronal plasticity, (neuro)steroids, and memory fluctuate over endogenous hormonal milieu, it is likely that these factors covary.

In summation, the present data indicate that neuro(active) steroids, in particular neurosteroids THP and DHEAS, can produce activational effects on spatial/reference, working, and long-term memory in females. Whether these neurosteroids affect performance, storage, or retrieval aspects of memory has not been ascertained. These neurosteroids' effects appear memory specific because they have effects in different memory assays, dose-dependently and independent of motoricity (McGaugh, 1989a). Although dose-dependency was not exhaustive and dosages employed were supraphysiological, these preliminary data indicate a causal relationship between neurosteroids and females' spatial/reference, working, and long-term memory that warrants further investigation. These findings have implications for fluctuations in neuroplasticity, neuro(steroids), and memory function over the estrous cycle and other reproductive states (Rossmanith, Reichelt, & Scherbaum, 1994). These data may help yield an effective model to better understand steroids' mechanism of action and functional role upon cognition. Further investigations, ascertaining neurosteroids' role on memory in different hormonal and reproductive states, may elaborate upon sex differences in incidence of cerebrovascular accidents and intensity of memory deficits exhibited in patients in the early stages of Alzheimers.

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