Progesterone After Estradiol Modulates Shuttle-Cage Escape by Facilitating Volition

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ABSTRACT: In animal models of depression, depression is defined as performance on a learning task. That task is typically escaping a mild electric shock in a shuttle cage by moving from one side of the cage to the other. Ovarian hormones influence learning in other kinds of tasks, and these hormones are associated with depressive symptoms in humans. The role of these hormones in shuttle-cage escape learning, however, is less clear. This study manipulated estradiol and progesterone in ovariectomized female rats to examine their performance in shuttle-cage escape learning without intentionally inducing a depressive-like state. Progesterone, not estradiol, within four hours of testing affected latencies to escape. The improvement produced by progesterone was in the decision to act, not in the speed of learning or speed of escaping. This parallels depression in humans in that depressed people are slower in volition, in their decisions to take action.

KEYWORDS: learning, volition, learned helplessness, depression

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Introduction

Some features of depression in humans can be modeled in nonhumans. These models of depression use learning tasks performed in the laboratory to define the degree of depressive-like symptoms that are produced in an animal. To understand the convergence of nonhuman animal models of depression, learning, and the potential modulation of these by ovarian hormones, summaries of three key areas are needed. Described here first is information about some of the characteristics of depression in humans. Next is a description of the establishment of the validity of nonhuman animal models of depression. Finally, a brief review of work on the role of ovarian hormones in nonhuman animal models leads to several unanswered questions about the roles of estrogen and progesterone in learning that are the basis of the research described here.

A few characteristics of depression that are important to highlight include differences between the sexes, fluctuation of symptoms with ovarian functioning, and characteristic psychomotor functioning. The incidence of depression among human females in their reproductive years is at least twice that in human males. Symptoms of major depressive disorder and less-severe transient changes in mood are associated with fluctuations in hormones during the menstrual cycle, after birth, and with menopause.[1,2] Concomitant cognitive features of major depressive disorder include, among other findings, deficits in psychomotor functioning, short- and long-term memory, and decision-making. Depressed people, as compared to nondepressed people, have no deficit in sensing stimuli nor do they have a deficit in executing a chosen motor action.[3–7] Their psychomotor retardation stems from a longer time to make a decision to act, a process termed volition, "the implementation of an intention to act" (p. 251).[8]

Overt behavioral aspects of depressive symptoms have been modeled in rodents by measuring the impact of an initial exposure to an inescapable mild shock. In one type of follow-up to the inescapable shock, rodents are given the opportunity to escape a shock in a different circumstance. The previous experience of lack of control makes subjects less likely to learn to escape when they do have control. This paradigm, learned helplessness, stemmed from the work by Mowrer and Viek in the 1940s and by Seligman, Overmier, Maier, and colleagues in the 1960s and 1970s.[9–11] The paradigm inspired vigorous dialog and gained momentum because it (1) proposed a cognitive mechanism of the learning phenomenon and (2) was applied to human depression.[12] Although much caution must be used when applying results to the human condition, the technique appears to have good validity in reproducing some specific and general behavioral features of depression as well as

the concomitant physiological changes, including depression's covariation with the fluctuations of or withdrawal from ovarian hormones.[13–16]

Animal models of depression define depression as performance on a learning task. There are three phenomena of interest in this model. The first is the induction of the depressive-like state, learned helplessness. The other two, closely related to each other, are learning that escape from an aversive stimulus is possible and then the actual performance of that escape response in what is known as a shuttle cage. The slower the escape in the shuttle cage, which is reflected in a longer latency, the greater the indication of depressive-like symptoms. All the three phenomena (induction of helplessness, learning, and performance) may be separately influenced by ovarian hormones. Although helplessness can be reliably induced in male rats, it has been less reliably induced in female rats.[17–19] In studies of naturally cycling female rats, results indicate that either performance was unrelated to the estrous phase[20] or ovarian hormones buffered females from the helplessness induction.[21] However, the lack of a reported effect on the performance of naturally cycling females is somewhat surprising. The effect of ovarian hormones on escape have been demonstrated in other aversive tasks.[22–46] No experimental data are available to assess the relationship between ovarian hormones and learning to escape electric shock without helplessness induction.

This paper addresses the effects of ovarian hormones on aversive escape learning and performance without helplessness induction. We view this issue as essential to answer before proceeding to more complicated questions concerning the effects of ovarian hormones on learning after helplessness induction. Given the importance of the escape paradigm in the nonhuman animal model of depression and widely documented influences of ovarian hormones in other tests of learning, it is essential to know the influences of ovarian hormones in escape learning to put into perspective escape learning after helplessness induction.

We test several hypotheses in order to clarify the roles of estradiol and progesterone in shuttle-cage escape learning without prior helplessness induction. Data from quasi-experimental studies suggest that although ovarian hormones may buffer helplessness induction, those hormones do not affect escape latencies.[20,21] If this is true, then ovariectomized females will perform well in the escape task and their performance will not depend on hormone replacement that they are given. If hormones do influence learning without helplessness induction, then, contrary to prior assertions, females in different hormonal states should perform differently in the escape task.

In light of the large body of literature on the effects of ovarian hormones on learning, estradiol and progesterone are expected to affect shuttle-box escape learning independent of helplessness induction. Several more hypotheses can be advanced regarding the direction of effects of ovarian hormones. First, because estrogens, typically 17β-estradiol, are known to increase activity[47] or because of a potential anxiolytic effect,[48–50] estradiol may cause escape latencies to be shorter because animals' arousal levels are not too high to interfere with learning and performance. Second, and in contrast to the first, because estrogens are known to increase adrenal responsiveness to adrenocorticotropic hormone and potentially make worse the effects of stress,[51–53] estradiol may cause escape latencies to be longer. Third, because progesterone or a metabolite of progesterone is known to have anxiolytic or antidepressant effects,[14,54–62] progesterone alone may cause escape latencies to be shorter. Fourth, if progesterone does reduce escape latencies, because estradiol upregulates progesterone receptors,[63] progesterone following estradiol so as to mimic estrus may cause escape latencies to be the shortest of all possibilities tested.

Subcutaneous injections of ovarian hormones given to ovariectomized females followed by testing in a shuttle cage will allow evaluation of these hypotheses. Examination of the change in subjects' behavior over trials will allow some insight into whether hormones are affecting differently acquisition of the escape response as compared to performance.

Method

Subjects and housing. Seventy-five female Sprague-Dawley rats were purchased from a supplier (Charles River Laboratories) and had been ovariectomized at 21 days of age. Food (Harlan Teklad 2016) and water were available continuously upon arrival in the laboratory, each female lived individually in a clear plastic cage measuring 43 cm deep, 21 cm wide, and 20 cm high. The solid bottom was covered by Sani-Chips bedding (P. J. Murphy Forest Products) and the top with a stainless steel wire lid. Cages and water bottles were cleaned every two weeks. The colony room was on a 14:10 light:dark cycle with lights off at 11:30 AM. Room temperature was maintained between 20°C and 23°C. All testing took place during the dark phase of the light cycle in a separate room, adjacent to the colony room, that was dimly lit by a 40-W desk lamp. The testing protocol was approved by the St. Bonaventure University Institutional Animal Care and Use Committee.

Apparatus. Latency to acquire an escape response was tested in a rat shuttle cage (Habitest; Coulbourn Instruments) with inner dimensions of 51.5 cm × 25.2 cm × 28.9 cm (W × D × H). The side walls were of shorter dimension and were made of stainless steel. The front and back walls were clear plastic; the front wall was a door clasped at the top and hinged at the bottom. The floor was made of 36 0.4-cm diameter stainless steel rods arranged in parallel, 1.55 cm apart, spanning the short dimension of the cage. Inside the chamber, a stainless steel wall bisected the long dimension to divide the chamber into two roughly square compartments. In the center of the bisecting wall, an opening—9.5 cm wide and 8.1 cm high—allowed the animals to move from one half of the chamber to the other. Five parallel photoelectric beams were in each half of the chamber, spaced 3 cm apart, with the

first one 2 cm from the center wall that divides the chamber. Thus, as an animal moved from one side of the chamber to the other through the opening in the bisecting wall, the pattern of interrupted photoelectric beams could be used to track the location of the animal as being in either half of the chamber.

A desktop computer ran software (Graphic State 3.0, Graphic State Notation; Coulbourn Instruments) to control all the events in the chamber and recorded a rat's location as being in the left or right half of the chamber. A 0.6 mA scrambled shock could be delivered to the grid floor from a shock generator (Coulbourn Precision Programmable Animal Shocker; Coulbourn Instruments).

Ovarian hormones. Estradiol (β-estradiol-3-benzoate; Sigma-Aldrich) and progesterone (Acros Organics) were dissolved in commercially available peanut oil at concentrations of 20 and 500 µg/cc, respectively. Dosage was 20 and 500 µg/kg (modeled after Ref. 43 and modified based on our experience with different doses of estradiol). This resulted in injections of 0.1 cc of oil for every 100 g of body weight.

Design. To examine the hypotheses regarding the roles of estradiol and progesterone in shuttle-box escape learning, ovariectomized rats received injections of either of the two hormones or just the oil vehicle. Injections were at 48 and 4 hours before the start of testing in the shuttle cage (Table 1). For rats receiving estradiol at 48 hours and then progesterone at 4 hours (E–P, $n = 12$), this produced sexual receptivity like the day of estrus in naturally cycling females. To determine the effects of progesterone without estradiol, some females (V–P, $n = 13$) received the oil vehicle at 48 hours and then progesterone at 4 hours before testing. To determine the effects of estradiol without progesterone, some females (E–V, $n = 12$) received estradiol at 48 hours and then oil vehicle at 4 hours before testing. To determine the importance of the order of hormone and, to an extent, a state of the estrous cycle in which progesterone had declined and estradiol had increased, some females (P–E, $n = 12$) received progesterone at 48 hours and estradiol at 4 hours before testing. Finally, to determine the effect of any exogenous hormone, some rats (V–V, $n = 12$) received oil vehicle at both 48 and 4 hours before testing. Data from 14 animals were excluded from analysis because power outages or equipment problems caused a loss of data. Thus, 61 rats were tested successfully within 21 days of ovariectomy.

Table 1. Hormone replacement injections.

GROUP	HOURS BEFORE TESTING	
	48	4
E–P	Estrogen	Progesterone
V–P	Oil vehicle	Progesterone
E–V	Estrogen	Oil vehicle
P–E	Progesterone	Estrogen
V–V	Oil vehicle	Oil vehicle

Escaping from mild electric shock requires moving from one side of the shuttle cage to the other via the small opening at the center of the wall dividing the cage into two chambers. Behavioral testing is done in two phases.[17,18,20,21,64] The first phase, FR1 (fixed response), requires one shuttle once the shock has begun. As soon as the shuttle is completed, the shock is terminated. If the animal fails to shuttle within 30 seconds, the shock is terminated automatically. After five such trials with intertrial intervals varying randomly from 30 to 90 seconds, the second phase follows immediately. In the second phase, FR2, two-shuttle responses are required. If the animal fails to shuttle two times within 30 seconds, the shock is terminated automatically. After 25 such trials with intertrial intervals varying randomly from 30 to 90 seconds, the testing ends. The first phase is traditionally limited to five trials because this task is learned easily. The second phase is harder to learn and more effectively differentiates groups with different learning abilities.

Procedure.

Hormone replacement. Subjects were weighed and the appropriate amount of oil vehicle alone or of oil containing an ovarian hormone as indicated by the test condition was drawn into a syringe. The oil was injected subcutaneously in the caudal half of the trunk along the dorsal midline.

Testing. Animals were transported with their living cage to the adjacent testing room. They were placed into either the left or right half of the testing chamber, side of placement counterbalanced within each hormone treatment condition. The software running on the desktop computer controlled all the events and timing and recorded latencies between onset of shocks and shuttling between the sides of the chamber.

Data analysis. For any trial on which a subject did not meet the shuttle criterion within 30 seconds, a default latency of 30 seconds was assigned for that trial. For each subject, means of the 5 FR1 trials and means of the 25 FR2 trials yielded one latency per subject, per schedule (FR1 or FR2). Data were also examined for trends across the five FR1 trials and across blocks of several FR2 trials, as described in the "Results" section. For each subject, percentage of default trials was calculated and examined for differences among treatment conditions. Latencies from default trials were then excluded, and the latencies for successful escapes were analyzed. Between groups or mixed-model ANOVAs were used in each analysis. For all analyses, two-tailed criterion for statistical significance was that $P \leq 0.05$. Fisher's protected t was used for post hoc comparisons between groups and was reported as an absolute value. Tukey's honestly significant difference (HSD) was used for post hoc analysis of a repeated measure.

Results

Latencies collapsed across trials. For the five trials requiring one-shuttle response (Fig. 1, left panel), mean escape latencies per group were fairly short (6.4 seconds overall) and did not differ among the five hormone treatment groups ($F_{4,56} = 1.25$, $P = 0.30$).

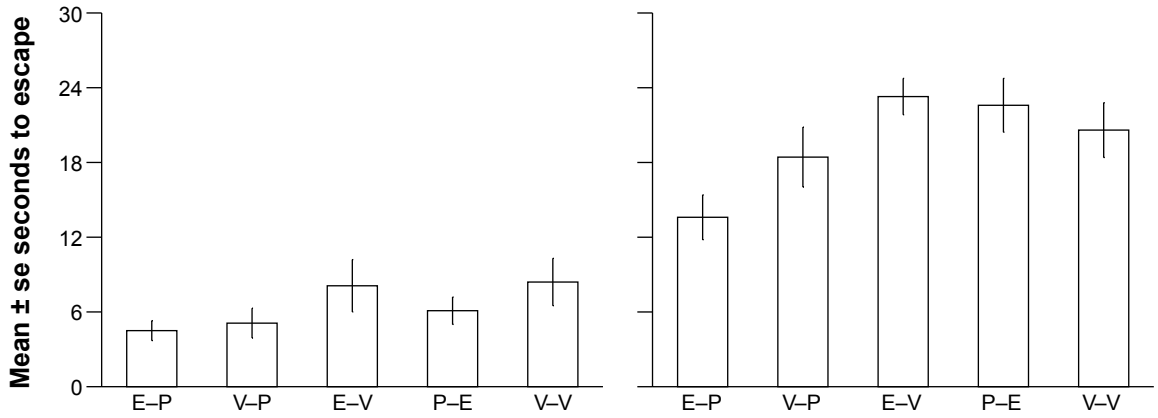

Figure 1. Mean (±se) latency in seconds to escape shock for animals in each condition. Left panel: mean of five trials, each requiring one-shuttle response (FR1). Right panel: mean of 25 trials, each requiring two-shuttle responses (FR2).

For the 25 trials requiring two-shuttle responses (Fig. 1, right panel), mean escape latencies were more than three times longer (19.7 seconds overall) as compared to the trials requiring one-shuttle response ($F_{1,56} = 247.05$, $P < 0.001$) and were different among the five hormone treatment groups ($F_{4,56} = 3.51$, $P = 0.01$). Subjects receiving estradiol and then progesterone (E–P) 48 and 4 hours before testing, respectively, escaped more quickly than did those subjects who received estradiol and then vehicle (E–V), progesterone and then estradiol (P–E), or vehicle for both injections (V–V), Fisher's protected *t*-test: all ts ≥ 2.38, all Ps ≤ 0.05. Subjects receiving vehicle and then progesterone (V–P) were intermediate between E–P and the other groups and were not significantly different from any of them: Fisher's protected *t*-test: all ts ≤ 1.68, all Ps > 0.05.

Latencies by trial or trial block. For the five trials requiring one-shuttle response (Fig. 2, left panel), mean latency to escape across all groups was more than doubled from trial 1 (3.3 seconds) to trial 5 (8.8 seconds: main effect of trials: $F_{4,224} = 6.59$, $P < 0.001$), and this pattern was not different for the five groups (no interaction of group by trials: $F_{16,224} = 0.95$, $P = 0.51$).

For the 25 trials requiring two-shuttle responses (collapsed into five blocks of five trials each; Fig. 2, right panel), the pattern across blocks depended on hormone group (interaction of group by block: $F_{16,224} = 1.87$, $P = 0.03$). Although latencies in the E–P group dropped slightly from block 1 to 5 (14.27–12.37), the mean latencies for the other four groups did not decrease but either increased across trials or started relatively high and stayed high. In fact, the latencies for the group receiving no hormones increased across trials (17.63–22.98, Tukey's HSD, $P < 0.05$).

Percent of trials with default latencies. The previous analysis of mean latencies included the default latencies of 30 seconds when animals failed to meet the shuttle requirement. This is the type of analysis that has been reported historically.[20,21] That kind of analysis can be misleading. Close inspection of the raw data suggested that on each trial subjects either escaped fairly quickly or failed to shuttle the required

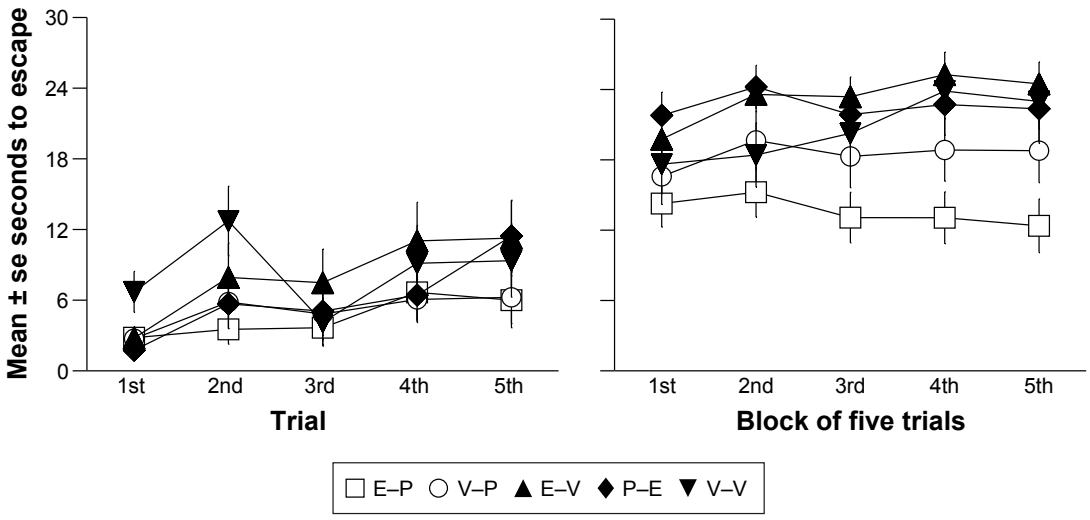

Figure 2. Mean (±se) latency in seconds to escape shock for animals in each condition. Left panel: each of the five trials required one-shuttle response (FR1). Right panel: each of the 25 trials required two-shuttle responses (FR2); trials are averaged into five blocks of five trials each.

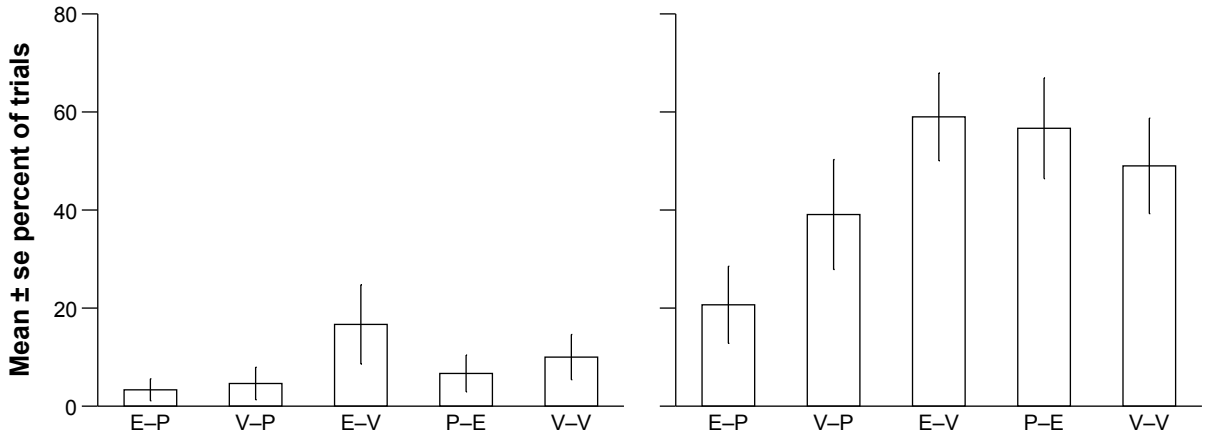

Figure 3. Mean (±se) percent of trials with default latencies of 30 seconds, indicating that animals failed to shuttle within the required time. Left panel: percent of default trials for the five trials that required one-shuttle response (FR1). Right panel: percent of default trials for the 25 trials that required two-shuttle responses (FR2).

number of times within the 30-second limit. Thus, the next step in the analysis was to determine whether groups differ in the frequency of default trials.

For the five trials requiring one-shuttle response, groups did not differ in the percent of trials on which subjects failed to shuttle during the 30-second trial (Fig. 3, left panel; $F_{4,56} = 1.23$, $P = 0.31$).

For the 25 trials requiring two-shuttle responses, the percent of trials on which subjects failed to shuttle two times during the 30-second trial depended on the hormone treatment (Fig. 3, right panel; $F_{4,56} = 2.54$, $P = 0.05$). Subjects in the E–P group had fewer default trials than did the subjects in the E–V group or subjects in the P–E group (Fisher's protected t-test: both ts ≥ 2.21, both Ps ≤ 0.05. The V–P and V–V groups were intermediate between but not significantly different from the E–P group on one hand and E–V and P–E groups on the other hand.

The pattern of defaults is more striking when viewed as a percentage of trials per 5-trial block (Fig. 4). The hormone treatment groups appear to diverge by the end of the FR2 phase. Although it appears that the E–P and V–P groups have separated from the other three groups, which rise across blocks, the interaction of hormone treatment group × block was not significant ($F_{16,224} = 1.33$, $P = 0.18$).

Latencies without default trials. To determine if there were effects of hormones on latencies for the trials in which animals did shuttle successfully, default trial latencies of 30 seconds were excluded from the calculation of mean latency to escape and these nondefault latencies analyzed. For trials requiring one-shuttle response, only one animal was excluded from the analysis because it did not have at least two nondefault trials (E–P, $n = 12$; V–P, $n = 13$; E–V, $n = 11$; P–E, $n = 12$; V–V, $n = 12$). For trials requiring two-shuttle responses, only individuals with at least five trials in which they shuttled successfully within the 30 seconds were included in the analysis (E–P, $n = 12$; V–P, $n = 10$; E–V, $n = 10$; P–E, $n = 9$; V–V, $n = 10$). Escape latencies for neither the one-shuttle task (Fig. 5, left panel) nor the two-shuttle task (Fig. 5, right panel) varied according to hormone treatment group (one shuttle required: $F_{4,55} = 1.58$, $P = 0.19$; two shuttles required: $F_{4,46} = 1.56$, $P = 0.20$). Mean (± se) latency for the trials requiring two shuttles, 12.3 (0.6) seconds, was more than twice than that for the trials requiring only one shuttle, 4.5 (0.6) seconds. This is not surprising because two shuttles were typically accomplished by entering the other chamber and turning around to go back to the original chamber.

Latencies, without default trials, by trial blocks. To examine trends across trials requiring two-shuttle responses for evidence of improving performance, default latencies of 30 seconds were excluded and the remaining trials were averaged into three blocks of 8, 9, and 8 trials each. This increase

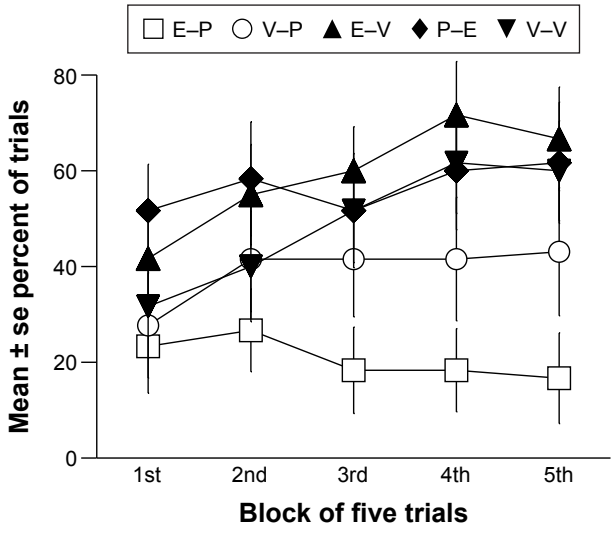

Figure 4. Mean (±se) percent of trials with default latencies of 30 seconds, indicating that animals failed to shuttle within the required time. Each of the 25 trials required two-shuttle responses (FR2); trials are averaged into five blocks of five trials each.

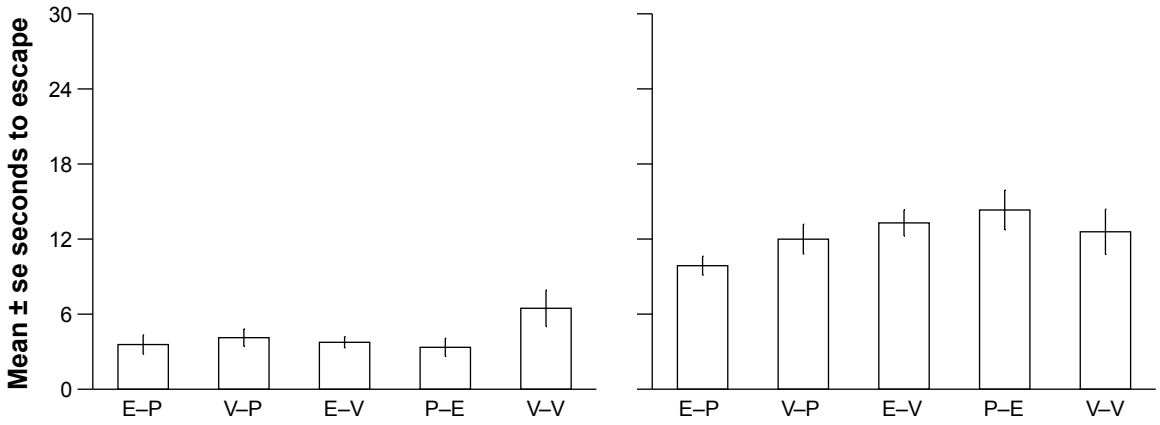

Figure 5. Mean (±se) latency in seconds to escape shock for animals in each condition after default latencies of 30 seconds were excluded from analysis. Left panel: mean of five trials, each requiring one-shuttle response (FR1). Right panel: mean of 25 trials, each requiring two-shuttle responses (FR2).

of the number of trials per block was necessary in order to include only those subjects who successfully shuttled at least one time in each of the three blocks (E–P, $n = 10$; V–P, $n = 10$; E–V, $n = 8$; P–E, $n = 7$; V–V, $n = 9$). Latencies were fairly constant across the three trial blocks (Fig. 6; $F_{2,78} = 0.16$, $P = 0.86$); this trend did not depend on the particular hormone treatment condition (block × condition interaction: $F_{8,78} = 0.93$, $P = 0.50$). The difference among the test conditions was short of significance ($F_{4,39} = 2.48$, $P = 0.06$). From Figure 6, a potential difference among conditions seemed to emerge in the last block of trials requiring two shuttles. The groups receiving progesterone within four hours of the start of testing, E–P and V–P, appear to perform slightly better than the other groups as reflected by shorter mean latencies, but differences among groups in this third block of trials was not significant (ts ≤ 1.84, Ps > 0.05). Of course, by the end of testing, the E–P and V–P groups had completed successfully (the opposite of defaulting) more trials than had the other groups and thus had received more negative reinforcement than had the animals in the other three groups. It is not known whether continued testing with the other three groups to the point where they successfully completed as many trials as the other animals would have resulted in slightly shorter latencies, supporting the interpretation that hormones facilitated the acquisition of the behavior.

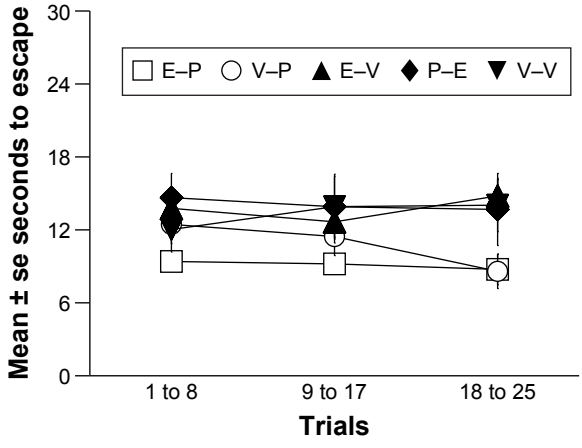

Figure 6. Mean (±se) latency in seconds to escape shock for animals in each condition after default latencies of 30 seconds were excluded from analysis. The 25 trials requiring two-shuttle responses (FR2) were divided into three blocks of 8, 9, and 8 trials each.

Discussion

Replacement ovarian hormones given to ovariectomized female Sprague-Dawley rats had a clear effect on behavior in the shuttle-cage escape task. This effect appeared to be the result of progesterone given four hours before testing when estradiol preceded it by 44 hours, a regimen that mimics the hormonal condition of estrus and produces the behavioral response of lordosis. Progesterone increased the likelihood that an ovariectomized female would initiate escape action. This seems parallel to the description of volition in humans.[8] Animals in every hormone treatment group could escape equally quickly, but a lack of progesterone administration made it less likely for them to try. Clearly, progesterone did not have an analgesic effect,[65] which could have lead to greater tolerance of the shock and slower latencies.

In contrast to those animals receiving progesterone within four hours of testing, females who were given only estradiol or given no hormone replacement performed relatively poorly in the escape task. Although estradiol is reported to increase activity,[47] estradiol alone did not shorten mean latencies or make escape action more likely.

The pattern found here might be inconsistent with at least one other study[21] that found rapid escape performance with five estrous and five diestrous (progesterone would be lower on than on estrous day) Holtzman female rats tested in an escape paradigm very similar to that used in the current study, five FR1 trials followed by 25 FR2 trials. After other females experienced helplessness induction one hour before the shuttle-box escape task, however, Jenkins et al did find that estrous females were faster

than diestrous females. In contrast to that difference between estrous and diestrous females reported by Jenkins et al, another study using the same strain of rat and a 24-hour delay between inescapable shock training and escapable shock testing did not find that rats in estrus either during training or testing were any different from rats at other stages in their cycles; they were all impaired in the escape task.[20] Clearly, more work is needed to address the influence of strain of rat, interval between helplessness induction, and hormone replacement on escape learning. The results of the study presented here certainly suggest that an effect of hormonal state on shuttle-box escape can happen even without helplessness induction. This suggests that caution should be used in interpreting the effect of hormones as helplessness buffering. Hormones can affect performance independent of helplessness induction. Our study tested rats during the dark phase of the light cycle, a time that they would normally be more active. However, other studies tested rats often during the light part of the cycle, a time that they would normally be less active.

The benefit of progesterone found in the current study of shuttle-box escape learning is parallel with the findings of studies of escape from a Morris water maze. Of course, a water maze is typically used to assess spatial learning ability, but it does not mean that the results of that task may not also reflect the influence of hormones on learning to escape a stressful situation. Progesterone alone or in combination with estrogen improves performance in water mazes.[23,28–30,32,34–38] Any beneficial effect of progesterone may come after its conversion to a metabolite[31,66] and through that metabolite's agonistic action at $GABA_A$ receptors,[67] which are inhibitory in adult mammals. It should be noted, however, that not all studies have found progesterone to benefit escape in a Morris water maze.[39–41,43–46,68]

There was no clear evidence that hormones affected the speed of learning the escape response. In the phase requiring one-shuttle response, latencies for all groups were similar and, surprisingly, increased across trials (as found in Ref. 17). Such an increase contrasts with the expectation that learning would be reflected by decreasing latencies. The increased latencies suggest a change in ability to effectively perform the task if subjects had, in fact, learned the task. In the phase requiring two-shuttle responses, latencies decreased only in the group receiving both estradiol and then progesterone afterward, but that decrease was minimal, 14.27–12.37 seconds. Thus, for this phase as well as the first phase of testing, replacement hormones may have affected differences between groups in the performance of the escape behavior. This apparent effect on performance becomes more obvious when differentiating between trials in which animals escaped and those on which they did not escape within 30 seconds.

The effect of progesterone, following estradiol in particular, seems to manifest itself as the decision to take action. It is remarkable that the analysis of only those trials on which animals successfully shuttled indicated that latencies did not differ among the hormone treatments. When animals responded to the shock by shuttling the required number of times, they all did so equally quickly. Acquisition of the task was apparently accomplished early in training because changes over trials were minimal and groups did not differ. This suggests that the difference among hormone groups is in the initiation of action and not in the sensing of the uncomfortable shock, the speed of learning of the required response, or the speed of execution of the required response. This is comparable to results from forced swim task indicating that progesterone or its metabolites can reduce the number of periods of immobility without affecting the speed of activity in general.[57,59] The lack of responding, immobility, for example, is interpreted to reflect a behavioral aspect of depression. Progesterone seems to reduce immobility or inactivity in a learning task that is used as a probe in an animal model of depression. The results and interpretation reported here are also consistent with the possibility that progesterone has an antianxiety effect[14,54–57,60–62] or antidepressive effect.[58,59] Moreover, the results obtained here with rats parallel the notion that depression in humans is a disorder of volition, the failure to make a decision to take an appropriate action.[8] It is important to note, however, that helplessness was not induced in our subjects. Nevertheless, the pattern of responding in the escape tasks resembled subjects in which helplessness had been induced[21] and suggest that a clearer understanding of hormonal influences on shuttle-cage escape is needed before interpreting effects of hormones as buffering helplessness. Those hormones may simply affect performance whether helplessness is or is not induced.

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Author Contributions

Conceived and designed the experiments: SMT and DJM. Analyzed the data: DJM, SMT, SMC, and MJF. Wrote the first draft of the manuscript: DJM. Contributed to the writing of the manuscript: DJM and SMT. Agreed with manuscript results and conclusions: DJM, SMT, SMC, and MJF. Jointly developed the structure and arguments for the paper: DJM and SMT. Made critical revisions and approved the final version: DJM, SMT, SMC, and MJF. All the authors reviewed and approved the final manuscript.

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