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The University of Vermont



Department of Psychological Science

Undergraduate Honors Thesis

Impacts of Estrogen and Progesterone Replacement During Acquisition on Habit Formation in Ovariectomized Female Rats

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May 2021

Table of Contents

Abbreviation List.....	3
Abstract.....	4
Introduction.....	5
Methods.....	8
Results.....	14
Discussion.....	18
Conclusion.....	24
Acknowledgements.....	24
Works Cited.....	25

Abbreviation list

Dopamine (DA)

Dorsal medial striatum (DMS)

Dorsolateral striatum (DLS)

Estrogen (E2)

Estrogen and Progesterone (E2+P)

Habitual (RD-insensitive)

Lithium chloride (LiCl)

Ovariectomized (OVX)

Reinforcer devaluation (RD)

Stimulus-response associations (S-R associations; habits)

Variable-interval schedule (VI s)

Variable-time schedule (VT s)

Abstract

Behavioral motor outputs transition from goal-directed to habitual following extended instrumental training, and is related to increases in dopamine (DA) release in the dorsal striatum. DA signaling in this region is positively correlated with circulating estrogen (E2) levels. Previous studies in our laboratory used lithium chloride (LiCl) reinforcer devaluation (RD) to identify habitual behavior in ovariectomized (OVX) female rats, and found that replacement with E2 resulted in goal-directed responding after a level of training where intact females express habitual behavior. The present study was designed to determine whether ovariectomized female Long Evans rats with estrogen *and* progesterone (E2+P) replacement during acquisition demonstrate habitual behavior following the same level of training. Results indicate that E2+P replacement supports the development of habitual (RD-insensitive) responding in female rats. These data suggest that the cyclic variation of *both* estrogen and progesterone throughout the estrus cycle and, possibly, their impacts on dorsal striatal DA underlie the sex-differences seen in the formation of habit.

There are two forms of instrumental learning: goal-directed actions and habits. For goal-directed actions, the association between an action and the rewarding value of its outcome drives behavior. Through repeated exposures of an action paired with an outcome in a specific learning environment, goal-directed behaviors can become habits. This occurs when the behavior becomes linked to contextual stimuli instead of being based on the value of the reward and the behavior occurs almost involuntarily (Adams, 1982; Dickinson, 1985). These behaviors are also known as stimulus-response (S-R) associations or habits. Habitual behaviors may be adaptive by promoting efficiency and decreasing cognitive resources needed to perform a task, or maladaptive if a harmful behavior such as addiction becomes habitual. However, it is important to note that once tasks have been relegated to habits, these behaviors can be inhibited, and goal-directed behaviors can be reinstated under certain conditions.

The brain processes information to produce motor outputs in two distinct learning and memory regions of the basal ganglia. Humans and animals rely on these pathways to process reward information and regulate instrumental learning. Both of these pathways involve different portions of the dorsal striatum which produces motor behavior via projections to the globus pallidus and thalamus (Haber, 2016; Tepper, Abercrombie, & Bolam, 2007), along with the brain regions that innervate the striatum.

One of these regions has been found to regulate goal-directed outputs and involves the dorsal medial striatum (DMS). Lesion studies have shown that the DMS is necessary for the acquisition and expression of goal-directed behavior (Yin, Ostlund, Knowlton & Balleine, 2005). The rewarding value of an action-outcome association is essential to producing a goal-directed behavior, however, the rewarding value of the association can be disrupted by interference with goal-directed processes.

The other region involved in motor learning is regulated by the dorsolateral striatum (DLS) has been found to control S-R associations (Graybiel & Grafton, 2015; Jog, 1999; Macpherson, Morita, & Hikida, 2014; Packard and Goodman, 2012; Packard & Knowlton, 2002; Yin, Knowlton, & Balleine, 2006). Lesions to the DLS resulted in DMS control over behavior and a continuation of goal-directed responding after reward devaluation (Yin & Knowlton, 2006).

Goal-directed and habitual behavior can be detected in the laboratory with reinforcer devaluation (RD) procedures. During these procedures, reward value is disrupted through taste aversion conditioning where the reinforcer becomes paired with injections of LiCl - which induces nausea. Because goal-directed responding is driven by the reinforcing value of the outcome, animals that are goal-directed are expected to decrease responding for the reinforcer in response to a reduction in its value (Adams & Dickinson, 1981). Conversely, habitual behavior is insensitive to RD (Adams, 1982) and can be identified by a continuation of responding following RD.

Previous studies in the Toufexis laboratory conducted on adult female Long Evans rats have found that females transition from goal-directed to habitual behavior at an earlier point in training (between 120-160 reinforcers; Schoenberg et al., 2019) than adult male Long Evans rats who show habitual behavior when exposed to 360 response-reinforcer pairings (Dickinson et al., 1995). This sex difference in the amount of instrumental training it takes for females vs males to demonstrate habitual behaviors may be attributable to the difference in circulating gonadal hormones.

Acute estrogen rapidly increases dopamine within the DLS of female rats (Yoest et al., 2018). Since the dorsal striatum is heavily innervated by dopamine-releasing neurons, it is sensitive to changes in DA levels (Everitt & Robbins, 2013; Haber, 2014). However, estrogen has also been shown to disrupt DLS mediated stimulus-response learning while enhancing

hippocampal place learning (Korol and Wang, 2018), leading us to hypothesize that a high estrogen state would maintain goal-directed behavior in female rats. To test this, we administered proestrus-level 17- β estradiol (E2) in a pulsatile fashion - to mimic cyclic E2+P levels across the 4-day estrus cycle - during a regimen of instrumental training that we previously showed to support habit formation in intact female rats (Schoenberg et al., 2019). We found OVX rats, both with and without E2 replacement, demonstrated goal-directed behavior following this degree of training, suggesting that the advanced habit formation observed in intact females compared to males is probably not solely an estrogenic effect (data not yet published).

This was not surprising since cycling estrogen is closely followed by cycling progesterone throughout the estrous cycle in intact females. Progesterone has been shown to interact synergistically and antagonistically with estrogen to accentuate DA release or reverse (Callier et al., 2001; Palermo-Neto & Dorce, 1990; Dornellas et al., 2021). Therefore, we decided to test if, in addition to E2 replacement in OVX females, replacement with progesterone as well would impact habit formation. Based on the results from our previous experiment with E2 replacement alone, we hypothesized that if progesterone were acting antagonistically to estrogen, rats would demonstrate continued goal-directed behavior. However, if progesterone were acting to further enhance E2 stimulated DA release, we hypothesized that E2+P replaced OVX rats would maintain habitual responding at a level of training where intact females behave habitually.

Methods

Animals

36 adult Long Evans, previously-OVX female rats (Charles River, Quebec; 90 days old at the time of arrival) were housed in pairs in a climate-controlled colony room maintained at 23°C with a 12-hr light-on-light-off cycle (7:00 a.m. to 7:00 p.m.). All testing occurred during the light phase of the cycle. All rats were given five days following arrival in the colony room to habituate before being gently handled to become accustomed to all experimenters. Following recovery from surgery, rats were put on restricted feed to maintain a target weight of 85% of their ad libitum weights for the duration of the experiment to ensure rats were motivated to earn sucrose pellet reinforcers during training. This was done by weighing rats daily and calculating the amount of food needed to maintain their target weight. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont.

Estrogen and Progesterone Treatment

All rats were assigned to a hormone treatment group: Low E (control) or E2+P. All rats underwent a noninvasive surgery to implant 10mm porous silastic capsules containing 5% 17- β estradiol (E2; Tocris) and 95% cholesterol (Sigma Aldrich) subcutaneously at the scruff of their neck (Almeij et al., 2013). These capsules have been shown to produce a consistent circulating concentration of E2 of ~20 pg/ml (Mannino et al., 2005), which is within the range observed in the diestrus phase of the estrous cycle (Overpeck et al., 1978), and were given to all animals in order to ensure estrogen receptors remained present despite ovariectomy. Low levels of E2 have not been found to effect response learning (Korol and Wang, 2018). Rats then received either an injection of proestrus level E2 in oil (10 ug/kg) and 0.05mL of progesterone (Sigma Aldrich) into the scruff of the neck (E2+P group) or an equivalently sized injection of oil (Low E group) 12 hours before the

first session of variable-interval nose-poke acquisition and again 12 hours before the fourth session of acquisition to simulate rising and falling levels of hormones seen in the natural rat estrus cycle.

Instrumental Training

Apparatus

The training apparatus consisted of six standard rat operant chambers (Med Associates, St. Albans, VT) kept within individual noise-attenuating cabinets. In the center of the right-facing chamber wall is a head-entry port into which a hopper delivers a 45-mg sucrose pellet (Bio-Serv). To the right of the head-entry port is a nose-poke device (ENV-114, Med Associates) that emits an infrared beam. When animals perform a nose-poke, this beam gets disrupted and signals for the delivery of a sucrose pellet to the operant chamber based on the schedule of reinforcement being used. All data from the operant boxes was monitored and collected by MED-PC software (Med Associates). Each rat was assigned to a single operant chamber at the beginning of the experiment in which they were trained and tested for the duration of the experiment. The house light in the chambers was illuminated at the beginning of each session, and automatically turned off at the termination of each session.

Magazine training

All rats received two 30-min sessions of magazine training, during which sucrose pellet reinforcers were presented on a variable-time 60-s (VT 60-s) schedule. During these sessions, the nose-poke device was physically removed from the operant chambers to allow for the animals to become familiar with the operant chambers and learn the positive value of the sucrose reinforcer, as well as the sound of the sucrose pellet being delivered to the magazine.

Nose-poke acquisition

Following magazine training, all rats received two sessions of training with a continuous schedule of reinforcement, where every nose-poke response was reinforced, for a total of 25 reinforcers earned over the course of each session. During these two sessions, rats learned the association between the nose-poke response and receipt of sucrose pellet reinforcement. Animals then underwent 4 sessions (1 per day for 4 days) of nose-poke acquisition training on a variable-interval 30-s (VI 30-s) schedule. Variable interval schedules have been shown to facilitate habit formation (Dickinson et al., 1983). These sessions terminated after each rat had earned 40 reinforcers, for a total of 160 response–reinforcer pairings over the course of the VI 30-s acquisition training. This degree of training was used because we have previously found intact female rats to demonstrate habitual behavior when trained to 160 reinforcers (Schoenberg et al., 2019).

Reinforcer devaluation (RD)

Following the final session of acquisition, half of the animals were randomly assigned to a Devalued group, with their cage-mates matched to a Non-Devalued group. All rats then underwent a reinforcer devaluation paradigm that consisted of a series of two-day cycles (Thrailkill & Bouton, 2017). During each session of RD, nose-poke responses were prevented by physical removal of the nose-poke holes from the operant chambers. On odd-numbered days in this paradigm, all rats were placed in the operant chambers, however, only the Devalued group received sucrose pellets (starting with a total of 40 pellets on Day 1). On these days, rats in the Non-Devalued group did not receive any sucrose, and their sessions were time-yoked to their Devalued counterparts. Upon the completion of each session, all rats were removed from the operant chambers and injected intraperitoneally (i.p.) with a 10 ml/kg dose of 0.15 M LiCl to induce nausea, then returned to their home cages. On even-numbered days, the same procedure was conducted, but only the Non-Devalued group received sucrose reinforcers, while the Devalued group was placed in the operant

chambers for the same duration as their yoked counterparts without receiving any sucrose reinforcers. Following the termination of these sessions, all animals received an i.p. injection of 0.9% physiological saline of equivalent size to the LiCl injections. Using this paradigm, exposure to the operant chamber, to the sucrose reinforcer, and to injections of both LiCl and saline were controlled for. The only difference between the Devalued and Non-Devalued groups was the pairing of the LiCl induced nausea with the sucrose reinforcer, which only occurred in the Devalued group. As devaluation continued, animals in the Devalued group consumed increasingly fewer pellets during their sessions on odd-numbered days, and the average number of pellets consumed was then presented the following day to the Non-Devalued group in their sessions. These two-day cycles proceeded until all animals in the Devalued group stopped consuming sucrose pellets. This criterion of zero consumption in the Devalued group is considered evidence of a successful conditioned taste aversion to sucrose reinforcers, and allows for the interpretation of responding at test to be evidence of responding in habitual behavior. This required a total of 12 days (or six cycles) of RD.

Testing for habitual behavior and confirming devaluation.

Extinction test

On the day following the last day of RD, sensitivity to RD was tested during a 12-min session under extinction conditions where the nose-poke holes were present in the chambers, allowing for responses to be made, however, responding did not lead to reinforcement. Nose-pokes per minute were recorded by the computer software. If nose-poking was still a goal-directed action, it was expected that the Devalued group would respond significantly less than the Non-Devalued group. If nose-poking was insensitive to RD and therefore habitual, it was expected that rats in the Devalued group would continue nose-poking at an equivalent rate to the Non-Devalued group.

Consumption test

On the day following the extinction test, successful devaluation of the sucrose reinforcer was assessed during a brief consumption test. During this test, 10 sucrose pellets were delivered on a VT 30-s schedule. Nose-pokes were again physically removed, and consumption of sucrose pellets for each animal was recorded. It was expected that animals in the Devalued group would associate the delivered sucrose pellet with nausea and would avoid consuming any sucrose pellets - evidence of successful taste aversion conditioning. Contrarily, animals in the Non-Devalued group were expected to consume all delivered pellets. Animals in the Devalued group who failed to demonstrate successful taste aversion conditioning (consumed five or more pellets during the consumption test) were excluded from all analyses.

Reacquisition

On the day following the consumption test, successful devaluation was assessed again during a 30-min reacquisition test. Rats were placed in the operant boxes and again allowed to nose-poke for the reinforcer, which was delivered on a VI 30-s schedule, similarly to the initial acquisition sessions. During this session, the Devalued group should integrate the memory of the conditioned taste aversion to sucrose with the memory that the nose-poking response earns reinforcers. The Devalued group was, therefore, expected to exhibit decreased nose-poking (or a failure to reacquire) for the sucrose reinforcer as evidence of successful taste aversion conditioning. On the other hand, Non-Devalued groups, for whom the reinforcer maintains its value throughout RD, were expected to reacquire to baseline levels of responding.

Statistical Analyses

All data were subjected to Analysis of Variance. Average response rates per minute in acquisition were analyzed using repeated-measures ANOVA with a within-subjects factor of training session

and between-subjects dummy-coded factors of hormone treatment (Low E or E2+P) and anticipated devaluation group (Non-Devalued or Devalued). Response rates during the critical test in extinction were analyzed as a proportion of baseline response rates (response rates at test divided by response rates from the final session of acquisition: Nelson & Killcross, 2013; Shipman et al., 2018) in a two-way ANOVA, with dummy-coded variables for hormone treatment and devaluation group. Sensitivity to devaluation during the extinction test in each hormone treatment group was further analyzed using planned comparisons with estimated marginal means in order to assess sensitivity to devaluation in each hormone treatment group. Outlier performance at test was operationalized as two standard deviations above or below the group mean ($z = \pm 2$; Field, 2007), and these animals were excluded from all analyses. Another two-way ANOVA was employed to evaluate responding as a proportion of the baseline during the reacquisition test, and included dummy-coded variables for hormone treatment and devaluation group. Criterion for significance for all tests was set at $p < .05$.

Results

Two animals were excluded from all analyses: one animal in the Devalued group failed the consumption test (consumed more than five pellets) and one animal was an outlier at test ($z=2.13$).

The final n in each group is depicted in Figure 3.

Acquisition

Results from repeated-measures ANOVA indicated that there was a significant main effect of session during acquisition ($F(3,90)=93.59, p<.001$), confirming that all animals acquired the behavior of nose-poking for sucrose reinforcers (see Figure 1). There was no main effect of hormone treatment or anticipated devaluation group ($F's\leq 1$), indicating equivalent acquisition rates across all groups.

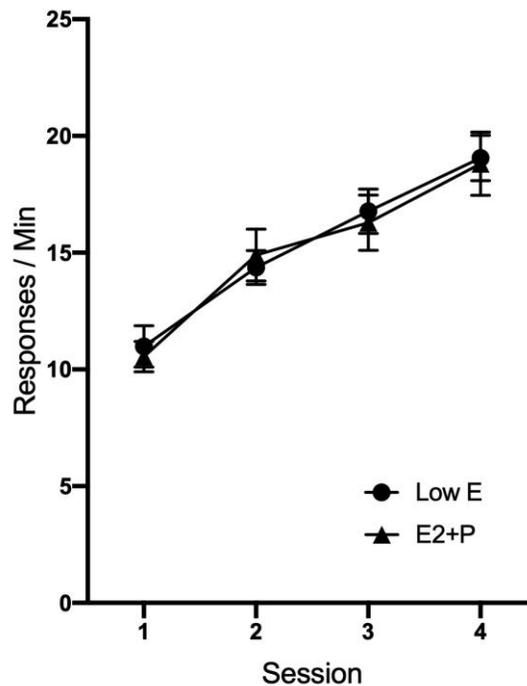


Figure 1. Average responses per minute across four VI 30-s sessions differentiated by hormone treatment groups. Error bars represent SEM.

Reinforcer Devaluation

All animals in the Devalued groups reached criterion of zero consumption of sucrose pellets by the end of RD (see Figure 2). RD took six cycles, or a total of twelve days of alternating LiCl and saline injections. All animals in the Non-Devalued groups consumed all delivered pellets on each saline day.

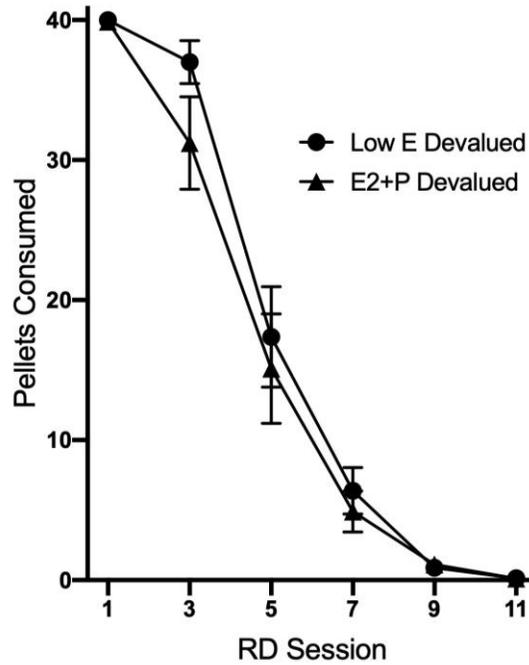


Figure 2. Mean number of sucrose pellets consumed by Devalued groups during sessions of RD.

Error bars represent SEM.

Extinction Test

Results from ANOVA revealed a marginally significant main effect of devaluation group ($F(1,30)=3.51, p=.071$). Planned comparisons showed that within the Low E hormone treatment group, the Devalued group significantly reduced responding compared to the Non-Devalued group ($F(1,30)=4.78, p=.037, \eta_p^2=0.14$, see Figure 3). In contrast, within the E2+P treatment group, there was no statistical difference between the response rates in the Devalued and Non-Devalued groups ($F(1,30)=0.315, p=.646$, see Figure 3). No main effect of hormone treatment ($F(1,30)=1.77, p=.193$) or hormone treatment by devaluation group interaction ($F(1,30)=1.48, p=.233$) was found.

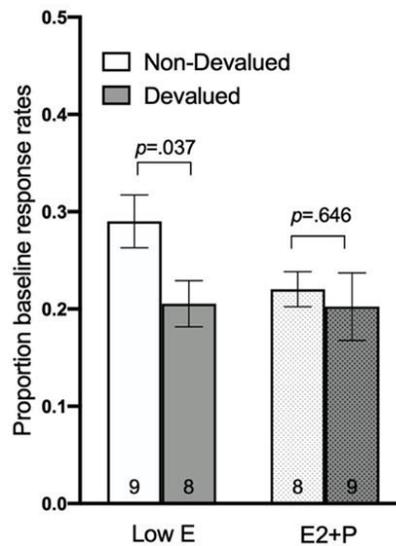


Figure 3. Mean responses per minute by the Devalued and Non-Devalued groups during the extinction test as a proportion of baseline. Error bars represent SEM.

Consumption and Reacquisition

One animal in the Devalued groups failed the consumption test and was excluded from all analyses. All other animals in the Devalued groups ate an average of zero sucrose pellets during the consumption test. All animals in the Non-Devalued groups consumed all delivered pellets as expected. In reacquisition, there was a significant main effect of devaluation group ($F(1,30)=124.97$, $p<.001$, $\eta_p^2=0.81$) such that both the Low E and E2+P Non-Devalued groups reacquired to baseline levels of responding, whereas the Devalued groups failed to reacquire (see Figure 4). Additionally, we did not find either an effect of hormone treatment ($F(1,30)=1.40$, $p=.247$) or hormone treatment by devaluation group interaction ($F(1,30)=1.24$, $p=.274$).

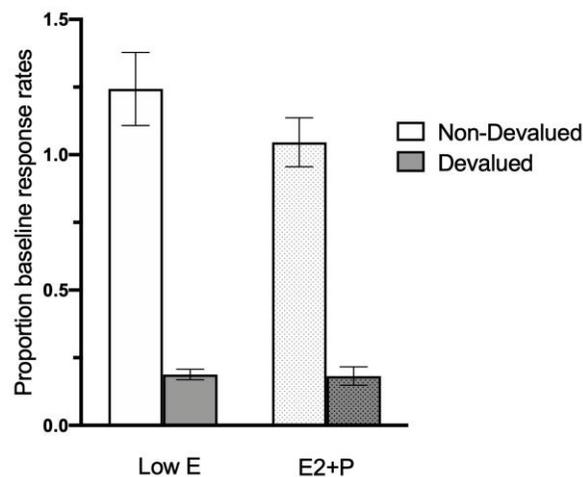


Figure 4. Mean responses per minute during the reacquisition test by Devalued and Non-Devalued groups as a proportion of baseline. Error bars represent SEM.

Discussion:

The results from this experiment demonstrate that, following a level of instrumental training where intact females are consistently found to respond habitually (Schoenberg et al., 2019), replacement with both E2 and progesterone during acquisition restores the acquisition of habitual behavior (as seen by the equivalent rates of responding in the E2+P Devalued group compared to the E2+P Non-Devalued group; Figure 3). The other results are important controls for confirming the efficacy of RD because successful taste aversion conditioning was verified in the consumption and reacquisition tests (Devalued groups failed to reacquire the nose-poke behavior while Non-Devalued groups reacquired to baseline levels of responding; Figure 4).

The transition from goal-directed to habitual behavior is accompanied by DA activation moving in the dorsal striatum through the DMS up to the DLS (Thorn et al., 2010; Haber, 2014). Increases in dopamine activity in the DLS along with the DLS' inhibition on the activity of the DMS have been found to play a role in driving an animal to express habitual behavior (Wickens et al., 2007; Belin et al., 2013; Haber, 2014). Psychostimulants such as cocaine and methamphetamine have been found to increase DA release in the dorsal striatum (de la Peña et al., 2015, Corbit et al., 2014; LeBlanc et al., 2013). Recently, results from another study conducted in our laboratory found that habit formation occurs in a sex-specific manner following methamphetamine treatment. Methamphetamine administered prior to instrumental training drives males to respond habitually at a level of training where they usually maintain goal-directed behavior. However, females that are trained to the level of habit are pushed back into goal-directed behavior (submitted to NBLM).

Similar to the effects of methamphetamine on DA neurotransmission, ovarian hormones such as estrogens in females have been found to influence striatal DA release. For example,

administration of E2 in vitro has been shown to significantly increase DA release in the dorsal striatum, though only in tissue from OVX rats, and not in tissue from intact males (Becker, 1990). Further, E2 administration led to a decrease in affinity of striatal D₂ DA binding sites in OVX rats after a single low dose of E2 (Levesque and Di Paolo, 1988), and again, this effect was sexually dimorphic and was only observed in OVX females, but not castrated male rats (Bazzett and Becker, 1994). Becker and Ramirez, (1981) found sex differences in striatal DA release in intact females in the estrous phase of the four-day estrous cycle. Females in the diestrous phase did not show these sex differences and had similar levels of DA release as males. OVX females showed significantly reduced DA release, however, treatment with E2+P to mimic the endogenous hormone release during the estrous phase restored the sex difference seen in intact animals (Becker and Ramirez, 1981). Estrogen receptors are not found on striatal DA terminals, but E2 indirectly influences DA signaling by releasing inhibition of GABAergic signaling on DA producing regions like the ventral tegmental area (VTA), which consequently increase DA release in the striatum (Becker, 2016 and Kokane and Perrotti, 2020).

While estrogen was suspected to play a critical role in the sex difference of habit formation, E2 alone does not appear to be responsible for this difference. When OVX females were administered proestrus levels of E replacement, groups remained goal-directed despite being trained to a number of reinforcers when intact females respond in a habitual manner (data not yet published). This finding may be explained by Korol and Wang (2018) who demonstrated that high estrogen states disrupt DLS mediated stimulus-response learning while enhancing hippocampal place learning and maintaining goal-directed behavior in dual solution mazes. Although place learning is not analogous to goal-directed behavior, the lack of RD-insensitive responding observed in E2 replaced OVX females may have been caused by an impairment of S-R responding.

New data presented here suggest that it is the cyclic fluctuation of both E2+P during acquisition that play a significant role in the sexual dimorphism evident in habit formation. It has been determined that ovarian hormones regulate changes in motivated behavior by influencing DA release. E2 treatment alone stimulated DA release, but E2+P treatment further enhanced DA release (Dluzen and Ramirez, 1984; Becker and Rudick, 1999). Yoest et al. (2018), demonstrated that DA release only slightly increases with treatment of either E2 alone for 4 days (with the last dose 24 hours prior to test) or progesterone by itself 4 hours prior to test without E2 priming. The enhanced DA release from E2+P treatment in a pulsatile manner that mimics the estrus cycle during acquisition could be the mechanism causing females to express habitual behavior earlier in instrumental training than males. This supports our hypothesis that progesterone is acting synergistically with estrogen to increase DA release.

Both E2 and P have been shown to have neuroprotective effects and have been studied in the context of neurodegenerative diseases like Parkinson's and schizophrenia. It has been suggested that the neuroprotective effects of progesterone are indirect in that they are mediated by one of progesterone's metabolites, Allopregnanolone (AlloP). AlloP is an inhibitory modulator of GABA_A receptors. A decrease in DA release was observed following AlloP inhibition on striatal GABA_A receptors (Briz et al., 2012).

The effects of AlloP on DA release in the nucleus accumbens, however, have been contradictory. When progesterone and, consequently, AlloP levels were high, phasic DA release decreased in the nucleus accumbens of male and female rats. The estrus cycle was found to modulate AlloP's effects in female rats, with those in proestrus (high endogenous progesterone levels) being less responsive to the effects of AlloP (Dornellas et al., 2021). Conversely Rouge-Pont et al. (2002), found AlloP to increase the release of DA in the NAc in male rats. The

differences in the results of these studies may be attributable to differences in the administered amounts of AlloP given to the treatment group in each study (7.5-25mg/kg to 12.5-100pmol, respectively). The 7.5-25mg/kg doses of AlloP were selected because they had been found to have neuroactive effects without producing sedative effects (Dornellas et al., 2021), while the 12.5-100pmol infusions of AlloP were low doses in the physiological nanomolar range (Rouge-Pont et al., 2002).

Another study on the effects of neonatal E2 treatment on stress-stimulated AlloP levels found that progesterone treatment reversed the increased DA release experienced during stressful situations caused by the neonatal E2 treatment in female rats (Procu et al., 2017). The role E2+P play on striatal DA release in sex specific manners is still ambiguous since many of these studies have been conducted solely on male mice. However, the reversal of effects of E2 treatments on DA release by progesterone via AlloP in female rats (Procu et al., 2017), and the results from Dornellas et al. (2012), that AlloP decreases DA release in the NAc, may both be affecting DA release in the striatum, and thus support our results from this study. Since we replaced with progesterone, we will need to conduct future studies with E2+AlloP replacement to determine if adding progesterone is dampening the effects of E2 replacement via this metabolite, or directly on progesterone receptors.

While our laboratory has been focusing on the dorsal striatum and its role in instrumental learning with the DMS being responsible for maintaining goal-directed behavior and the DLS controlling habitual behavior, the dorsal striatum does not exist in a vacuum and is not the only portion of the brain that has been implicated in instrumental learning. There are other regions within the medial prefrontal cortex that project to motor regions that have been found to influence habit development (Lingawi et al., 2016): the prefrontal cortex (goal-directed actions), and the

infralimbic cortex (IL; habitual behavior). Many of these regions are also innervated by dopamine releasing neurons that may be influenced by estrogen binding since estrogen receptors are found in many regions of the brain with regionally specific binding to DA receptors (Bazzett and Becker, 1994). Thus, estrogen and progesterone in E2+P administered during acquisition may have acted in cortical areas causing DA to increase in structures outside of the DLS - including the IL or PL (Lingawi et al., 2016).

A closer examination of our data reveals an interesting result: animals in the Non-Devalued (control) groups appear to respond differently depending on hormone condition. Animals in the E2+P Non-Devalued group responded at a lower rate than the Low E Non-Devalued groups. The detection of habit in the E2+P Devalued group is driven by the reduction in responding in the E2+P Non-Devalued group. It has been thought that habitual responding actively inhibits goal-directed responding and vice versa (Coutureau and Killcross, 2003), but each behavior is not permanent and goal-directed actions can be reinstated under necessary conditions. The results indicate that goal-directed actions are being suppressed in the E2+P Non-Devalued group, likely by another mechanism. This is different from what we normally see with habits because habitual responding is usually classified as an increase in responding despite reward devaluation due to RD-insensitivity.

This pattern of results - the apparent suppression of goal-directed responding in the Non-Devalued E2+P group - is similar to the results found by Coutureau and Killcross (2003), and Haddon and Killcross (2011), where inactivation of the IL was found to reinstate goal-directed responding in overtrained rats, indicating that the intact IL plays an important role in preserving habitual responding by inhibiting goal-directed behavior. The IL has been found to play a critical role in both the acquisition and expression of habitual reward-seeking behavior by actively

suppressing goal-directed behavior to encourage S-R learning and is directly targeted by dopaminergic neurons from the ventral tegmental area (Barker et al., 2014). Since DA modulation is influenced by E2+P in the striatum, further research needs to be conducted on the effects of E2+P on DA release in the IL. It may be that the fluctuations of these hormones act on the IL and encourage the suppression of goal-directed responding.

To study whether these sex differences in habit formation are due to structural differences that are set up early on during brain development, future research in this field should include adult castrated males given E2+P (to determine if it is the circulating ovarian hormones that cause females to behave habitually earlier in instrumental training); castrated newborn males (because testosterone begins circulating after birth); and newborn females given testosterone (since ovarian hormones are produced during adolescence).

In this experiment the purpose of the hormone treatment (E2+P group) was to determine the role these sex hormones play in the apparent sex difference in habit formation. Our results may have important clinical significance for menopausal women receiving estrogen replacement, since it is still unclear how estrogen and progesterone are involved in learning and the formation of habits. Those individuals may be susceptible to psychopathologies that are thought to involve dysregulated motor responses and addiction. Female rats have the ability to quickly become habitual (Schoenberg et al., 2019), but return to goal-directed control if central DA levels are increased (submitted to NBLM). However, the effects of these gonadal hormones on the expression of habit - administration of the hormones just before the extinction test - have not yet been studied, and might reveal differences in their effects.

Conclusion

In conclusion, we found that OVX females with E2+P replacement during acquisition express habitual behavior at the same level of training as intact female rats. We expanded on previous research to show that habit can be reinstated in overtrained OVX females with the replacement of both of these ovarian hormones. These findings are important in expanding our understanding of the role estrogen and progesterone play in instrumental learning systems in the brain, and how this contributes to the sex differences seen in habit development.

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