INVESTIGATING THE EFFECTS OF MEDROXY-PROGESTERONE AND HORMONAL BIRTH CONTROL ON HABIT BEHAVIOR IN FEMALE RATS

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Abstract

Recent research has shown that there is a significant sex difference in the rate of habit acquisition in rats, with intact females acquiring the behavior with fewer action-outcome reinforcer pairings than intact males. This difference is shown to be related to the presence of cyclic estrogen and progesterone during habit behavior acquisition training. Specifically, the presence of progesterone has been shown to be crucial in early habit acquisition. However, the mechanism of action through which progesterone is influencing this behavior is unknown. Additionally, this project explores the impacts of long-term hormonal (levonorgestrel - progestin based) birth control on habit behavior in intact female rats. There is a significant lack of research surrounding the cognitive and behavioral effects of hormonal birth control on the individuals utilizing it. This work begins to fill in this gap and hopes to increase awareness and understanding surrounding the greater impacts of hormonal birth control.

Experiment I of this thesis investigates how progesterone modulates this behavior acquisition through the administration cyclic high levels of estrogen and Medroxy-progesterone during habit behavior acquisition. Medroxy-progesterone is a form of progesterone that is not metabolized into neuroactive metabolites and augments the impacts of progesterone working only through the classic progesterone receptors. Habit behavior was not observed in the rats given this hormone treatment, thus implicating a different mechanism of action responsible for early habit behavior acquisition. It is hypothesized that a neuroactive metabolite, such as allopregnanolone, is imperative for early habit acquisition through its modulatory impact on $GABA_A$ receptors.

Experiments II and III examine the effect of levonorgestrel implants on habit behavior in intact female rats. Female rats were implanted with subcutaneous capsules that would slow-release levonorgestrel over the course of the experiment. Experiment II explores the impact of levonorgestrel on the acquisition rate of habit behavior. Female rats were undertrained, and they only received 100 action-outcome reinforcer pairings – the threshold for intact females to exhibit habitual responding is ~120 action-outcome pairings. The rats with levonorgestrel implants did not display habitual responding, suggesting that levonorgestrel does not accelerate the rate of habit acquisition. Experiment III investigates the effect of levonorgestrel on overtrained female rats, and if its presence would delay habit acquisition. The rats were trained to 200 action-outcome reinforcer pairings, where habitual responding has been consistently seen in intact females. However, the rats treated with levonorgestrel did not show habit behavior, thus indicating that the presence of levonorgestrel is delaying habit behavior acquisition.

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General Introduction

Habits are an integral part of our everyday lives and allow us to respond to context clues without dedicating specific thought to our action. They are the reason you can conduct daily tasks without thinking through each action, such as driving without fully paying attention to the streets you are turning onto, yet still arrive at your desired destination. Having habits allows for cognitive space to be freed up. The parts of our brains that would be thinking about how to respond to any given stimuli are able to focus on other, more pertinent, tasks (Lingawi et al., 2016).

Habits don't just spring up overnight – they develop from the repetition of goal-directed actions (Stalnaker et al., 2010). Goal-directed behavior occurs when you (or an organism in general) is motivated to conduct an action due to its expected outcome. This outcome is rewarding, and it reinforces the behavior. To use the driving example again, when you are driving to a new place of work for the first time, you are actively thinking about what streets to turn onto and where they will bring you. The safe and timely arrival at your destination is the rewarding outcome – you will likely take the same route to that place again, and again, and again. As these actions are conducted repeatedly, you begin to make new, contextual associations with the driving route. Perhaps you would learn that the big blue house on the corner means you should turn right, and you subconsciously start to associate the house with the action – the stimulus is now dictating your response.

The development of these stimulus-response associations is the acquisition of habit behavior. An important feature of habit behavior is that these stimulus-response behaviors persist, even when the outcome is not rewarding (Dickinson & Weiskrantz, 1997). Say you get in your car to go to the grocery store, and halfway along your drive

you realize that instead of going straight at the big blue house on the corner, you turned right and are now at your place of work. This outcome is not rewarding considering you did not want to be at work, however you habitually responded to the stimuli.

Habit behavior, specifically habit behavior acquisition, can be studied through instrumental training paradigms. This involves training an animal (commonly rats) to conduct a certain action (nose poke, lever press) through a reinforcing reward (often a desired food). As they repeat this action for the reward, the stimulus-response association is formed. They begin to associate the presence of the action device (nose poke hole, lever) with the behavior they are conducting, and they learn that the presence of the device means they should respond appropriately. To assess if habit behavior has been acquired, the animals will undergo outcome devaluation. Often, this takes the form of a conditioned taste aversion to the desired food reward which involves inducing gastric malaise following consumption of the reward (Dickinson & Weiskrantz, 1997). After devaluation, the animal is placed back in the context where it learned the behavior, and if their behavioral response rate is not significantly different than that of a non-devalued group, it is assumed they have acquired habit behavior. This is because if they were still in goal-directed behavior, their response rates would drop due to the absence of a rewarding outcome.

Habits are extremely important to our everyday functioning; however, they can also become maladaptive. The acquisition of habit behavior marks the loss of behavioral flexibility – once a habit is developed, the learned behavior is extremely hard to deviate from (Lerner, 2020). This adverse habit acquisition has been implicated in the development of certain psychopathologies, such as obsessive-compulsive disorders

(OCD), post-traumatic stress disorder (PTSD) or substance abuse issues (SA). The compulsions associated with OCD and the compulsive drug seeking that is integral to SA resemble habit behavior (Goodman et al., 2012). The repetition of these actions could result in their progression to a full-fledged psychopathology, with maladaptive habit behavior underlying its development. One potential reason for this could be stress response activation. It has been shown that many behavioral responses in stressful situations are likely derived from habit memory systems (Packard, 2009). Compulsions and drug seeking can both be categorized as anxiolytic (stress reducing) behaviors, and their repetition could be used as a soothing method. While their execution will temporarily relieve the stress, continued reliance upon them results in a psychopathology. The link between habit behavior and psychopathology development/perpetuation is under researched and not well understood, thus it is important to continue investigating this connection.

While studying habit behavior, it is important to understand the brain structures that underlie its acquisition. The dorsal striatum is implicated in being the primary modulator of goal and habit behavior. The posterior dorsomedial striatum (pDMS) is necessary for goal directed behavior to occur and lesions to the dorsomedial striatum have been shown to prevent action-outcome associations from occurring (Yin, Knowlton, et al., 2005; Yin, Ostlund, et al., 2005). Similarly, the dorsolateral striatum (DLS) is important in the development of habit behavior (Yin et al., 2004). Understanding the interplay in activity between the pDMS and DLS is critical to understanding the development and presentation of habit behavior. The infralimbic cortex (IL) is one area that is important in mediating the switch in activity between the pDMS and DLS. The IL

is located in the ventromedial prefrontal cortex (vmPFC) and facilitates the switch from DMS led behavior to DLS led behavior. The IL must be activated throughout habit acquisition training in order for habit behavior to occur. If the IL is inhibited throughout acquisition, habit is not observed (Smith et al., 2012; Smith & Graybiel, 2013). In sum, the DMS is necessary for goal directed behavior, while the DLS is necessary for habit behavior – and the change in activity between the two is facilitated by the IL cortex.

For many years, research surrounding habit behavior and habit behavior acquisition was only conducted in male animal models (Shansky & Murphy, 2021). This oversight has left a large gap in the literature surrounding many aspects of habitual responding in female rats. Recently, our lab found that there is a large sex difference in the rate of habit acquisition between male and female rats. It was found that intact female rats need significantly fewer action-outcome reinforcer pairings to show habit behavior, as compared to intact male rats (Schoenberg et al., 2019). As this difference was further investigated, it was determined that the early habit behavior acquisition could be largely attributed to the presence of cyclic estrogen and progesterone. The presence of progesterone is critical – when ovariectomized (OVX) female rats were given just estrogen, they displayed slower habit acquisition as compared to intact females (Schoenberg et al., 2022). Once progesterone was given, the OVX rats acquired habit behavior at a similar rate to the intact females. Overall, estrogen and progesterone are important mediators of a variety of different behaviors, and their combination has been shown to have important effects on striatal dopamine release (Becker & Ramirez, 1981a, 1981b). The presence of progesterone is shown to increase the amount of dopamine released, above and beyond that of just estradiol (Becker & Rudick, 1999). Dopamine is a

primary neurotransmitter throughout the brain and is necessary for goal directed actions to occur (Palmiter, 2008). Considering the striatum is integral to the acquisition of habit behavior, it is logical to infer that these changes in dopamine release and receptor regulation as a result of cycling hormone levels influence the behavior differences observed.

Progesterone, however, can affect the brain through a variety of different pathways. Progesterone can bind to its classical receptor, act through one of its neuroactive metabolites, or influence both pathways simultaneously. The classical progesterone receptors have been shown to have an expansive distribution throughout the brain and be involved in a variety of different processes (Zuloaga et al., 2012). Progesterone is also broken down into a multitude of neuroactive metabolites, which bind to a wide range of receptors and produce their own effects. A primary metabolite of interest is allopregnanolone. Allopregnanolone is one of progesterone's main neuroactive metabolites, and it binds to $GABA_a$ receptors where it exhibits positive, allosteric modulation. Considering $GABA_a$ receptors regulate neuronal inhibition, allopregnanolone thus has an inhibitory effect on the brain (Barbaccia et al., 1997; Liang & Rasmusson, 2018; Melcangi & Panzica, 2014). The mechanism of action that progesterone uses to modulate the early acquisition of habit behavior is not known.

While thinking about progesterone's large effect on the brain and its role as a modulator of behavior, additional queries arise – specifically, the impact that hormonal birth control has on the brain. Hormonal birth control was first introduced in the 1950s as an oral contraceptive pill (Pletzer & Kerschbaum, 2014). Birth control was a radical invention, and its introduction to society shifted the course of women's reproductive

rights. Women were given reproductive freedom and autonomy, which allowed them to further their education, careers, and have a lot more choice about when they wanted to have children (Planned Parenthood, 2015). The impact that birth control has had on the women's rights movement is undeniable, however minimal research has been conducted on the effects of birth control outside of contraception. The seminal research surrounding birth control's mechanism of action was done in animal models in the 1930s but since then, a majority of the work has been done in humans (Liao & Dollin, 2012). This has resulted in little to no knowledge surrounding the cognitive and behavioral impacts observed in individuals who take birth control.

Many of the hormonal contraceptives (HC) that are currently prescribed to individuals are progestin based, due to progesterone's ability to suppress the luteinizing hormone surge that causes ovulation and therefore prevent pregnancy. A primary progestin that is used in HCs is levonorgestrel (LNG). LNG is a synthetic progestin, and it binds to progesterone and androgen receptors – it does not bind to estrogen receptors (Lemus et al., 1992; Schindler et al., 2008). LNG is the progestin hormone that is slow released from all major intrauterine devices (the IUD – Mirena, Liletta, Skyla and Kyleena), and in the emergency contraception pill (Plan B) (Allen & Goldberg, 2007; Gemzell-Danielsson et al., 2021; Stovall et al., 2021; Whaley & Burke, 2015). It is extremely effective in its contraceptive abilities, however its impact on behavior and cognition is almost entirely unknown.

This research is attempting to close this knowledge gap and begin the conversation surrounding birth control's impact above and beyond its use as a contraceptive. This work will look at natural progesterone's mechanism of action in

regard to its influence on habit behavior acquisition and if the exogenous administration of synthetic progesterone (hormonal birth control) has an impact on habit behavior from two different angles.

Chapter I: Investigating the Effect of Medroxy-Progesterone on the Acquisition of Habit Behavior in Female Rats

Experimental Hypothesis

This research tested the hypothesis that progesterone is working through its classical receptor to mediate early habit behavior acquisition through the administration of Medroxy-progesterone. Medroxy-progesterone is a form of progesterone that is unable to be metabolized into neuroactive metabolites, such as allopregnanolone. Thus, the administration of Medroxy-progesterone assesses the classic progesterone receptor's impact on habit behavior acquisition. Cycling high levels of estrogen and Medroxy-progesterone were given to ovariectomized female rats during habit acquisition, and the rate of habit behavior development will be analyzed.

Materials and Methods

Subjects

16 female Long Evans rats from Charles River, Quebec were used in this study. Upon arrival they were 75-90 days old. All rats were maintained at 85% of their free body weight following ovariectomy surgery and before instrumental training.

Surgery: Ovariectomy and Silastic Capsule Implant

After a one-week acclimation period following arrival, all rats underwent ovariectomy surgery and were implanted with a silastic capsule. Each of these surgeries occurred at the same time. The subjects were anesthetized with isoflurane prior to

beginning surgery. A single incision was made dorsally, along the midline and below the final rib, and was approximately 1.5 centimeters long. The ovaries were removed bilaterally; internal incisions were closed with suture and external incisions were closed with wound clips. Following the ovariectomy, each subject was implanted with a subcutaneous silastic capsule at the scruff of the neck. Each capsule was 10 mm long and contained 5% 17β-Estradiol and 95% cholesterol. Following surgery, each subject was injected with 1.0 ml of Lactated Ringers solution to prevent dehydration, and one dose of an analgesic (carprofen (5.0 ml/kg)) immediately after, and 24 hours post-surgery. The rats were given one week to recover before being placed on a restricted food diet.

Hormone Delivery and Replacement

The subcutaneous capsule implant steadily releases a low (diestrus) level of estrogen that maintains estrogen receptor availability. Estrogen upregulates its own receptors, and without its presence, the receptors will disappear. The upregulation of estrogen receptors is the purpose of the subcutaneous capsule, and the estrogen is not at a high enough level to have a significant behavioral effect. All rats received subcutaneous injections of 10 ug/kg of E2, which was followed by 1.0 mg of Medroxy-progesterone 17-acetate (Millipore Sigma) dissolved in canola oil. These injections were given twice throughout acquisition; the night before the first day of variable-interval training, and the night before the fourth (final) day of variable interval training. This limits the hormone exposure to only the acquisition period and mimics the cyclical nature of ovarian hormones in intact female rats.

Training and Testing

Apparatus

The training apparatus contains six standard rat operant chambers that are housed within individual noise attenuating cabinets. The light in the chamber was turned on at the initiation of each session and automatically turned off at its end. The right wall of the chamber contains a head entry port in which a hopper delivers a 45-mg sucrose pellet. On the right of the head entry port, there is a nose poke device that emits an infrared beam. This beam is disrupted when the animal "nose-pokes" the device, and a response signal is recorded. MED-PC software collects the data from the boxes. Each rat was assigned a box in which they were trained and tested in throughout the entirety of the experiment.

Magazine Training

This training consisted of two, 30-minute sessions with the purpose of the rats familiarizing themselves with the operant boxes and learning the rewarding quality of the sucrose pellet. The nose poke device was removed from the box during magazine training. Sucrose pellets were delivered to the head entry port on a random time 60 second schedule.

Nose-Poke Acquisition

After magazine training concluded, the nose poke device was placed back in the operant box. The rats received two sessions of training with continuous reinforcement; each nose poke is reinforced with a sucrose pellet, until 25 pellets have been reached. This facilitates the learning of response and outcome associations (nose poke $\rightarrow$ sucrose pellet). Once this training has concluded, the rats receive four days of variable interval 30 second training (VI30). The rats are administered a total of 40 pellets that are delivered

unpredictably yet average out to be 30 second intervals. This results in the rats receiving a total of 160 response-outcome associations throughout acquisition.

Outcome Devaluation

After acquisition training, all of the animals were assigned to either the Devalued or Non-Devalued group. The nose poke device was removed from the operant box and sucrose pellets were delivered on a random time 30 second schedule. Outcome devaluation was conducted in two-day cycles which consisted of odd and even number days.

On odd days, all rats were placed in the chambers, however only members of the Devalued group received sucrose pellet reinforcers. The Non-Devalued group were only exposed to the chamber and did not receive sucrose pellets on odd number days. They were yoked to a counterpart in the Devalued group, and their operant box session ended when the member of the Devalued group had received all the pellets they were supposed to (day 1 began with 40 pellets). Following the session, all the rats were removed from the chambers, given an intraperitoneal injection of Lithium Chloride (LiCl) (10 ml/kg), and immediately returned to their home cage. LiCl induces nausea, thus conditioning those in the Devalued group to have an aversion to the taste of the sucrose pellet.

On even number days, the procedure stayed the same, however the rats in the Non-Devalued group received sucrose pellets. The Devalued group was yoked to the non-Devalued rats and were in the operant boxes for the same amount of time as their counterparts. Following the completion of these sessions, each animal was removed and immediately given an injection of 0.9% physiological saline interperitoneally. The saline injections were of a comparable size to the LiCl injections.

As devaluation continued, the Devalued rats consumed fewer pellets per session. The average number of pellets consumed on odd numbered days was the subsequent number of pellets given to the Non-Devalued group. Outcome devaluation continued on this two-day cycle until the Devalued group no longer consumed the sucrose pellets presented. The lack of consumption indicates that a taste aversion was successfully conditioned into the Devalued animals.

Test in Extinction

Devaluation sensitivity was tested through an extinction test that was conducted following the last devaluation day. Subjects were placed in the operant chambers for 12 minutes, and the nose-poke device was present. The number of times the responded to the nose-poke device was recorded through the MED-PC software. The responses were not rewarded with a sucrose pellet. The Devalued group continuing to respond to the device at the same frequency as the Non-Devalued group would indicate that they are responding habitually. The presence of the nose-poke device results in the rat continuing to respond to it, even though the outcome has been devalued.

Consumption Test

To test for proper devaluation, the rats were presented with 10 sucrose reinforcers on a variable interval 30 second schedule. The nose-poke apparatus was removed from the box during this test. It is expected that if successful devaluation occurred, that the animals in the Devalued group would not consume any of the sucrose pellets. Any animals that did not display proper devaluation were excluded from further analyses. Successful devaluation was marked by the animals consuming no more than 4 pellets during the

session. The criteria for this test were decided upon to allow for a margin of error from the machines, however, is also restrictive enough to ensure proper devaluation.

Reacquisition Test

Proper devaluation was tested again following the consumption test through a reacquisition test. Rats were placed in the chambers for 30 minutes, with the nose poke device, where sucrose pellets were delivered on a variable interval 30 second schedule. This test is examining the responding rates of the animals and comparing them to their baseline rates. The Devalued group is expected to have lower rates than their baseline, while the Non-Devalued group is expected to have comparable rates.

Statistical Analyses

For all analyses outlier exclusion criteria was operationalized as two standard deviations above or below the group means (z = +/– 2) A repeated measures ANOVA with a within-subjects factor of training session and a between-subjects factors of implant treatment (LNG vs. cholesterol) and devaluation group (Paired vs. Unpaired) was used to analyze acquisition rates (nose-pokes per minute) during the VI30 sessions. This was done to investigate if there were any significant differences in the rate of acquisition between groups. A significant difference was detected, and a further repeated measures ANOVA was conducted to analyze this. These baseline response rates were used in future analyses of response rate (extinction and reacquisition tests), specifically for response rate change. Reinforcer devaluation was confirmed using a repeated measures ANOVA with a within-subjects factor of session and a between-subjects factor of implant treatment. The number of pellets consumed by the Paired group in each implant treatment

group was the dependent variable. In cases where sphericity was violated (Mauchly's test was significant; ($p < .05$)), a Greenhouse Geisser ($\varepsilon > 0.75$) or Huynh-Feldt ($\varepsilon < 0.75$) correction was employed. The nose poke response rates during the extinction test were analyzed as a proportion of baseline (response rates during extinction test divided by the number of responses during the final acquisition session) in a two-way ANOVA with planned comparisons. Determination of the behavior being goal directed or habit was done through direct comparison between the Paired and Unpaired groups within each implant treatment. Reacquisition test response rates were analyzed as a proportion of baseline response rates using a two-way ANOVA including fixed factors of implant treatment and devaluation group. Criterion of significance for all tests was set at $p < .05$.

Experimental Timeline

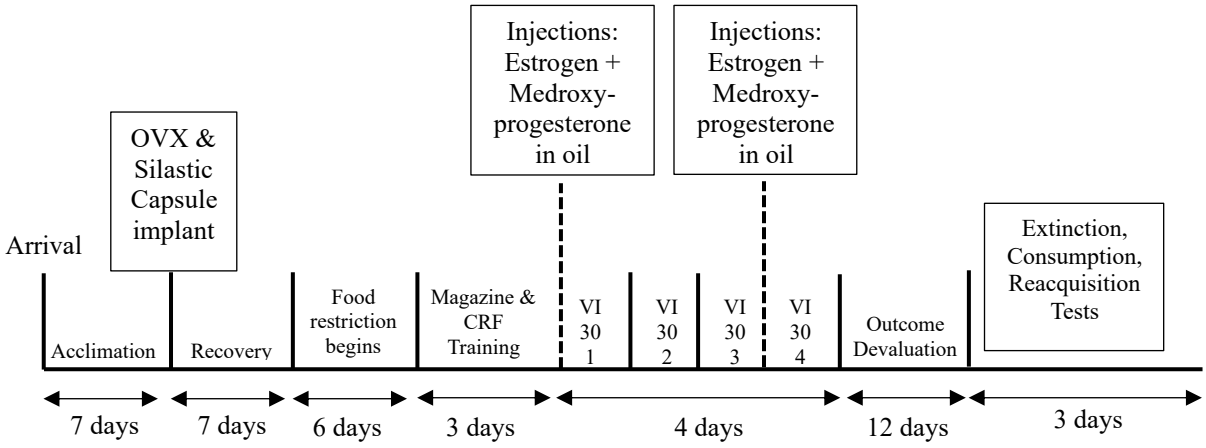

Figure 1. Experiment 1 timeline.

Results

Positive Control

The control results for this experiment are seen in Schoenberg et al. (2022), Experiment 1. The test in extinction results indicate that the control animals (ovariectomized, Long-Evans female rats) were exhibiting goal-directed responding at 160 reinforcers when trained under the same experimental paradigm (Figure 2).

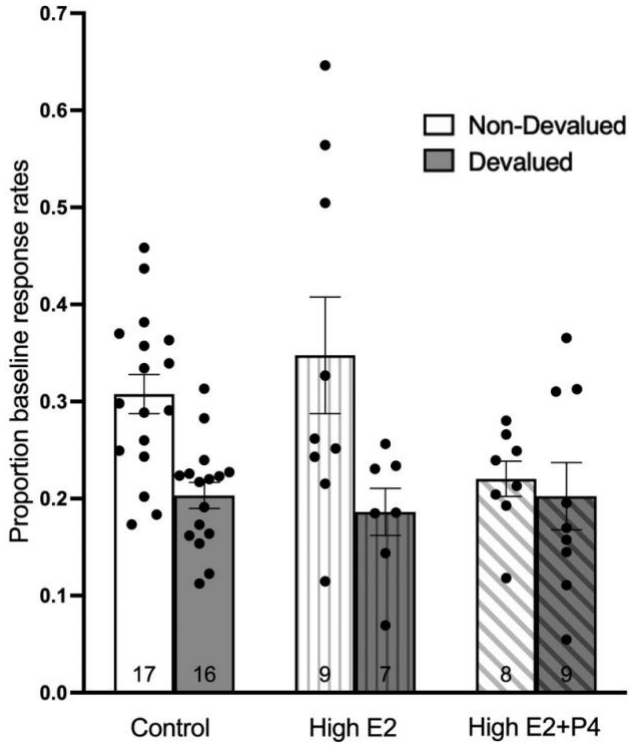

Figure 2. Response rates during the extinction test as a proportion of baseline. The Devalued Control and Devalued High E2 rats responded significantly lower than their Non-Devalued counterparts. The High E2 + P4 rats had no significant difference between rates of response in the Non-Devalued and Devalued groups. Numbers in bars represent final group n's. Error bars represent SEM.

Acquisition

Following ovariectomy surgery, one rat died and was not included in the analyses. Nose-poking for the sucrose reinforcer was acquired by all subjects and was confirmed to be significant by a repeated-measures ANOVA (significant within-subject factor of

session: $F(1.71, 23.95) = 37.47$, $p < .001$, $\eta p^2 = 0.73$). Acquisition rates were analyzed for the future devaluation groups, and there was no significant difference between groups (no significant effect of devaluation group: $F(1,14) = 0.40$, $p = .539$; no devaluation group by session interaction ($F(1.71, 23.95) = 0.31$, $p = .704$). There was no difference in acquisition rates between the subjects in the Devalued and Non-Devalued groups (Figure 3A).

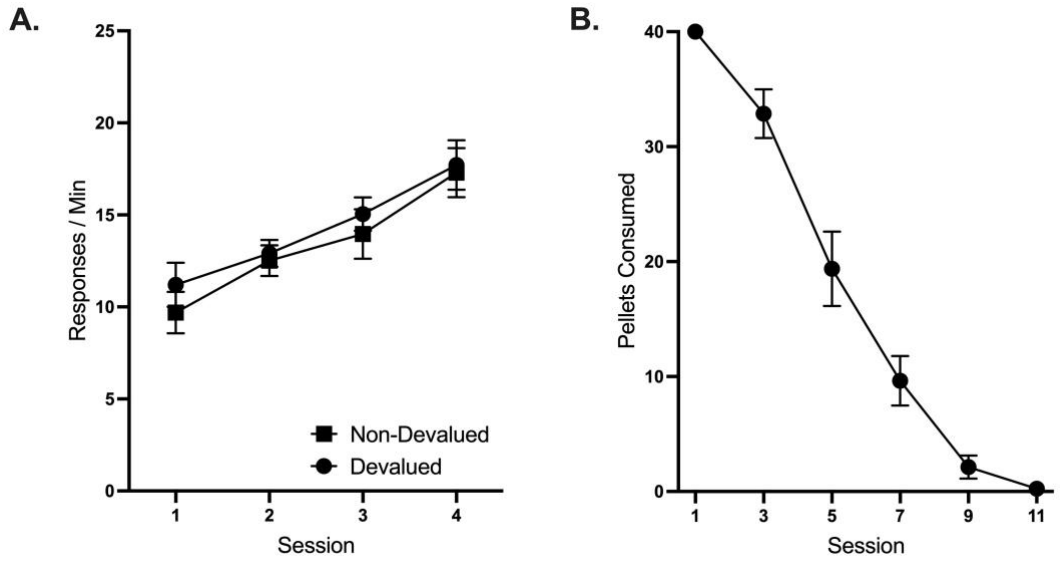

Figure 3. A. Nose-poke acquisition rate across VI-30-s sessions. **B.** Reinforcer consumption of the Devalued group throughout outcome devaluation. All subjects in the Devalued group reached zero consumption at the end of 12 sessions. Error bars represent SEM.

Outcome Devaluation

All subjects in the Devalued group reached zero consumption of sucrose pellets by the end of outcome devaluation (Figure 3B). All subjects in the Non-Devalued group continued to consume all sucrose pellets throughout devaluation (data not shown).

Test in Extinction

One animal was an outlier and was not included in further analyses (Devalued group, $z = 2.60$). The response rates of the Devalued group ($M = 0.21$, $SD = 0.06$) were significantly lower than the response rates of the Non-Devalued group ($M = 0.34$, $SD = 0.12$; $t(14) = 2.68$, $p = .018$, Cohen's $d = 0.09$). The significance of these results indicates that ovariectomized rats with cyclic estrogen and Medroxy-progesterone replacement during acquisition did not acquire habit behavior and remained goal directed (Figure 4).

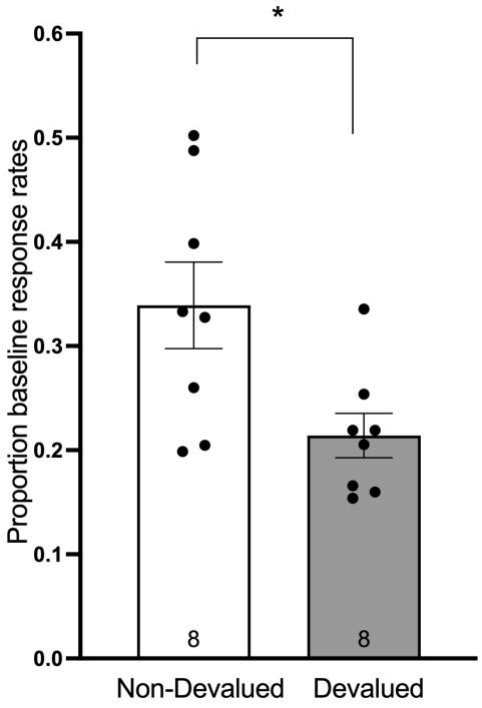

Figure 4. Response rates during extinction test as a proportion of baseline response rate. Response rates were significantly lower in the Devalued group than in the Non-Devalued group ($p = .018$). Numbers in bars represent final group sizes. Error bars represent SEM; * indicates $p < .05$.

Consumption and Reacquisition Tests

Proper outcome devaluation was confirmed by the consumption and reacquisition tests. The Devalued group ceased consumption of the sucrose pellets during the consumption and reacquisition tests, while the Non-Devalued group maintained consumption (data not shown). The reacquisition test showed a significant difference in response rates between the Non-Devalued and Devalued groups ($t(14) = 11.70$, $p < 0.001$, Cohen's $d = 0.18$; Figure 5). The Non-Devalued group maintained and exceeded their response rates as compared to baseline ($M = 1.20$, $SD = 0.22$), while the Devalued group had lower rates when compared to baseline ($M = 0.22$, $SD = 0.14$). The Devalued animals did not reacquire sucrose pellet seeking behavior.

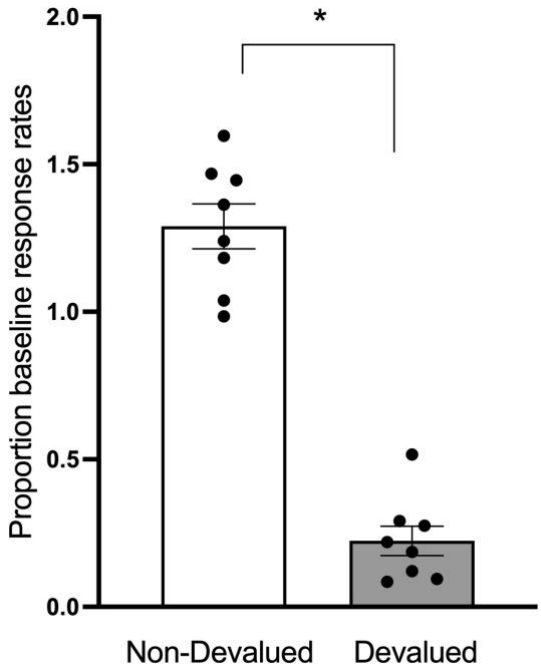

Figure 5. Reacquisition test response rates as a proportion of baseline response rates. Error bars represent SEM; * indicates $p < 0.05$.

Discussion

The results from this experiment indicate that ovariectomized rats administered cyclic high levels of estrogen and Medroxy-progesterone do not acquire habit behavior at an early stage (160 reinforcers, which is the number of reinforcer-exposures at which intact females usually exhibit habit behavior) (Schoenberg et al., 2019). Medroxy-progesterone is not metabolized into progesterone's metabolites and binds to the progesterone receptor at or above progesterone's own affinity (McAuley et al., 1993; Pridjian et al., 1987). Thus, the lack of early habit acquisition indicates that progesterone is likely not acting through its own receptor to mediate this behavior. Instead, we can assume that it is acting through one of its main metabolites. The primary metabolite of interest in investigating early habit behavior is allopregnanolone.

Allopregnanolone binds to $GABA_A$ receptors as a positive allosteric modulator and increases neural inhibition throughout the brain (Schumacher et al., 2014). Allopregnanolone is particularly influential on the δ-$GABA_A$ receptor subunit, many of which are located in the ventral tegmental area (Vashchinkina et al., 2014). The ventral tegmental area is a crucial part of the dopaminergic reward pathway, and alterations to this pathway can have drastic impacts on behavior. Allopregnanolone binding to $GABA_A$ receptors on dopaminergic neurons inhibits further pathway activation and impacts the influential effects of the reward pathway (Vashchinkina et al., 2014). Considering we know that dopamine has a large impact on habit behavior, it is logical to conclude that allopregnanolone's effects on dopaminergic neurons impact its acquisition.

The next step in further understanding the mechanism of action that progesterone uses to modulate early habit behavior is the direct administration of allopregnanolone.

This experiment's conclusion indicates that progesterone does not modulate early habit acquisition through the classical progesterone receptor, however this is not enough to truly understand what is occurring in the brain to produce the behavioral response. While allopregnanolone is the primary metabolite of progesterone, progesterone is metabolized into many neuroactive metabolites. The present research does not properly implicate allopregnanolone as a primary neural substrate responsible for early habit acquisition. Future research in this area should follow the same behavioral paradigm used in this experiment, however cyclic high levels of estrogen and allopregnanolone should be administered to ovariectomized rats. This is a crucial next step in understanding the nuances of habit behavior acquisition and progesterone's role in the rate of acquisition.

Chapter 2: Investigating the Effect of Levonorgestrel on the Early Acquisition of Habit Behavior in Intact Female Rats

Experimental Hypothesis

This research tested the hypothesis that levonorgestrel (LNG) would accelerate the acquisition of habit behavior. Rats were given subcutaneous capsules that contained LNG, designed to slow release over the course of the experiment which mimics the effect of long-acting reversible contraceptives. They were then trained with a minimal amount of training previously demonstrated to support goal-directed behavior. It is hypothesized that LNG will accelerate habit acquisition, considering it is a progestin and progesterone has been shown to modulate the early acquisition of habit in female rats.

Materials and Methods

Subjects

The subjects used in this study were 18 Long-Evans female rats. The rats were gonadally intact, and between 75-90 days. Following levonorgestrel capsule implant, all rats were maintained at 85% of their free body weight throughout training.

Estrus Cycle Tracking

All rats received daily vaginal swabs throughout the course of the experiment. Prior to implantation, the swabs were used to ensure that all the rats were cycling normally. Following capsule implantation, the swabs were done to ensure that the rats implanted with hormonal birth control had ceased cycling. Anovulation was determined

by a persistent diestrus state. Swabs were dipped in deionized and sterilized water, inserted into the vaginal canal, and the cells were smeared onto a glass microscope slide. Slides were scored before surgery and at the end of the experiment. Each day was labelled as proestrus (P), estrus (E), metestrus (M) or diestrus (D),. Proestrus was determined by the presence of predominantly nucleated epithelial cells, estrus by predominantly anucleated cornified cells, metestrus by proportional amounts of each cell type, and diestrus by predominantly leukocytes.

Surgery: Levonorgestrel Capsule Implant

All the rats underwent subcutaneous capsule implant surgery following acclimatization to the colony room (~ one week post arrival). The capsules were 3 centimeters long, made with silicone tubing and filled with either 30 mg levonorgestrel or cholesterol (control, TC). The rats were anesthetized with isoflurane, and each rat received two capsules implanted subcutaneously in the scapular region. The incisions were closed with wound clips, and each rat received injections of 1 mL Lactated Ringers (to prevent dehydration) and a 5.0 ml/kg dose of carprofen (analgesic) – they received one dose of carprofen immediately following, and 24 hours post-surgery. This methodology was developed using Hilz, 2022; Hilz et al., 2021; Ma et al., 2006. Seven rats received LNG capsule implants and eleven received cholesterol implants. This uneven split was due to not having enough LNG to make all the capsules.

Training and Testing

The training and testing methodology for this experiment was the same as experiment 1. There was one difference in the nose-poke acquisition training, specifically the variable interval 30 second training. The rats received four days of training, where they were delivered 25 pellets per training session on a variable interval 30 second schedule. They received a total of 100 reinforcers during acquisition, which does not produce habit in intact cycling rats.

Outcome devaluation was completed in 5 cycles (10 days), and devalued rats reached zero consumption in that time. Extinction, consumption, and reacquisition were conducted on the following days, following the experimental timeline.

Statistical Analyses

The same statistical analyses as experiment 1, with the same exclusion and correction criteria, were applied to this experiment. In addition, a Bayesian Factor analysis was conducted to quantify support for the null or alternative hypothesis over the other.

Experimental Timeline

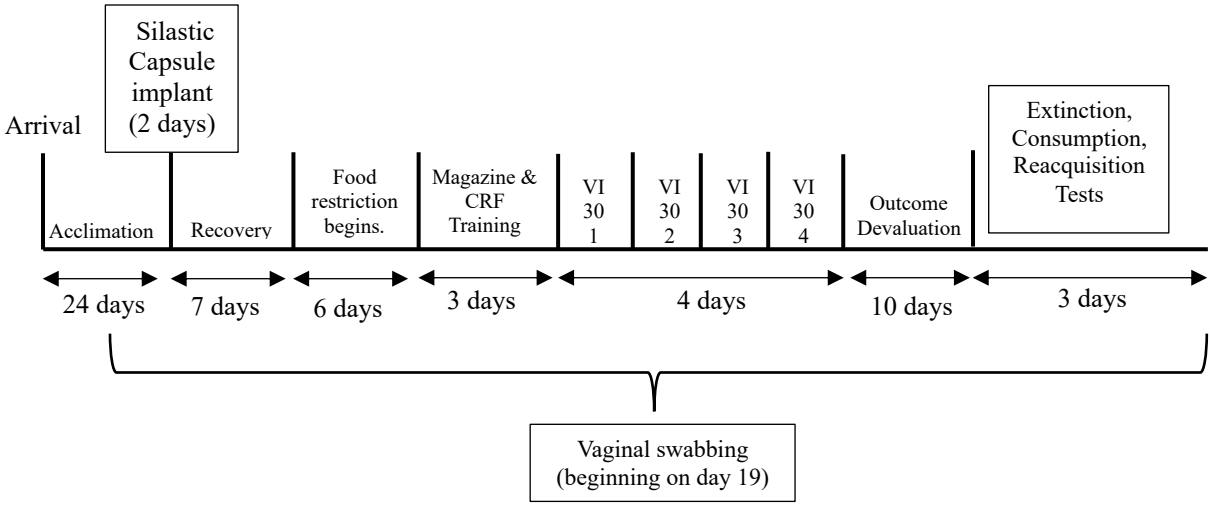

Figure 6. Experiment 2 timeline.

Results

Acquisition

The nose poke behavior was successfully acquired by all animals and response rates increased across the four VI30 sessions (25 reinforcers each, totaling to 100 reinforcers). During sessions 2 and 3, the LNG implanted rats had a significantly higher response rate than the cholesterol treated rats (Figure 7A2). A repeated measures ANOVA confirmed that there was a significant main effect of session ($F_{1.943,\ 25.26} = 21.44$, $p < .001$, $\eta_p^2 = .623$, $MSE = 11.52$, 95% CI [0.323, 0.742]). There was a significant effect of implant treatment ($F_{1,\ 13} = 7.89$, $p = .015$, $\eta_p^2 = .38$, $MSE = 25.87$, 95% CI [.02, 0.62]), however there was no significant effect of devaluation group ($F_{1,\ 13} = 3.21$, $p = .096$, $\eta_p^2 = .198$, $MSE = 25.866$, 95% CI [0, 0.495]) and no significant interaction effect ($F \leq 1$). These results indicate that all rats successfully integrated the nose-poke to sucrose reward

behavior, and that there is a marginal effect of implant treatment. Importantly, there was no significant difference between the anticipated devaluation groups (Figure 7A1).

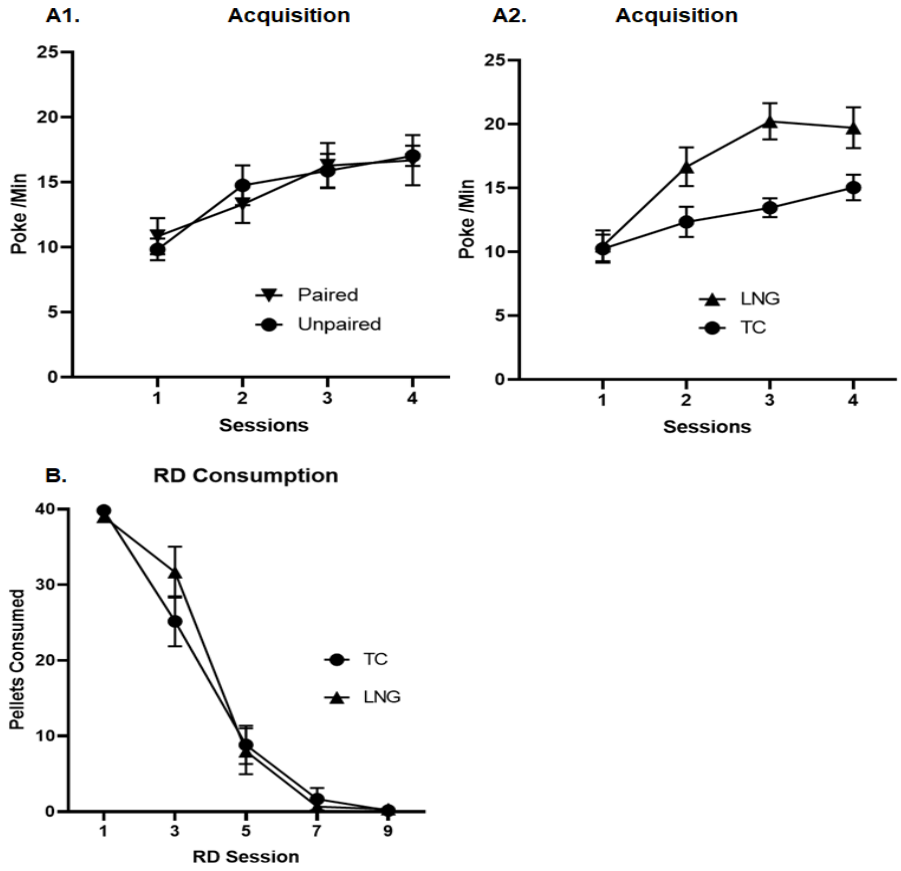

Figure 7. A1. Acquisition of nose-poke to sucrose reinforcer behavior by predicted devaluation groups. All animals show evidence of successful behavior acquisition. **A2.** Acquisition of nose-poke to sucrose reinforcer behavior by implant treatment groups. LNG treated rat responses were higher than cholesterol treated rats during sessions 2 and 3. All animals show evidence of proper behavior acquisition. **B.** Reinforcer consumption across outcome devaluation. All LiCL→ sucrose reinforcer paired rats decreased their consumption across devaluation sessions. Error bars denote SEM.

Outcome Devaluation

All animals in the paired LiCL→ sucrose reinforcer group reached zero consumption by the end of reinforcer devaluation (five, two-day cycles). The Devalued group successfully acquired a taste aversion to the sucrose reinforcer (Figure 7B). The

Non-devalued group did not attenuate their pellet consumption (data not shown). Mauchly's test of sphericity was significant (χ^2 (9) =23.221, p <.008), and a Greenhouse-Geisser correction was used (ε = .477). A repeated measures ANOVA was used to confirm successful devaluation ($F_{1.909, 13.364}$ = 166.996, p<.001, η_p^2 = .960, *MSE*= 30.79, 95% CI [.878, .97]). There was no significant main effect of implant (cholesterol or LNG) on the rate of devaluation ($F_{1, 7}$ = .148, p=.712, η_p^2 = .021, *MSE*= 48.314, 95% CI [0, .36]), nor a significant interaction effect (F≤1). This suggests that implant type did not influence devaluation rate.

Test in Extinction

One rat was determined an outlier and was excluded from further analysis. Analysis of the extinction test data revealed that there was a significant effect of devaluation ($F_{1, 13}$ = 23.46, p=.001, η_p^2 = .64, ω^2 = 0.599, *MSE*= .006, 95% CI [.023, 0.789]). The Non-Devalued cholesterol animals (M=.42, SD=.114), and the Non-Devalued LNG animals (M=.46, SD=.012) exhibited higher nose-poke responses than their Devalued counterparts, (M=.24, SD=.085) and (M=.25, SD=.059), respectively. There was no significant effect of implant treatment ($F_{1, 13}$ = .323, p=.579, η_p^2 = .024, *MSE*= .006, 95% CI [.0, 0.296]), nor a significant interaction effect between implant type and devaluation (F<1). A pairwise comparison with a *Bonferroni* correction was conducted and it showed that in the cholesterol treated rats, there was a significant difference in nose-poke response behavior between the Devalued and Non-Devalued animals ($F_{1, 13}$ = 11.82, p=.004, η_p^2 = .476, $Mean_{diff}$=.177, 95% CI [.066, 0.688]). This indicates that these animals are exhibiting goal-directed behavior (Figure 8). Further

analysis of the Bayes factor indicates that there is 3.06 times the indication of the cholesterol implant data occurring in support of the alternate hypothesis (goal directed behavior), with a .013% error ($BF_{10} = 3.06$). Additionally, the same analysis was done for the LNG treated rats and it revealed that there was a significant difference in nose-poke response behavior between the Devalued and Non-Devalued animals ($F_{1, 13} = 11.806$, $p = .004$, $\eta_p^2 = .476$, $\omega^2 = .42$, $Mean_{diff} = .177$, 95% CI [.066, 0.688]). This indicates that these rats are also displaying goal-directed behavior (Figure 8). A Bayes-Factor analysis was conducted and showed that there is 27.65 times the evidence of the LNG capsule implant data occurring in support of the alternative hypothesis, goal-directed behavior, with a .00033% error ($BF_{10} = 27.65$).

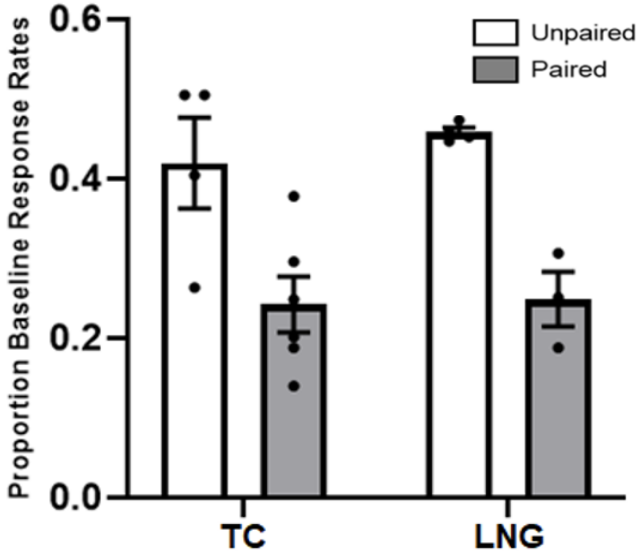

Figure 8. Extinction. Response rates during a non-reinforced extinction test, as a proportion of baseline response rates. All rats, regardless of implant treatment, in the paired group responded significantly less than those in the unpaired group. Error bars denote SEM.

Consumption and Reacquisition Tests

Outcome devaluation was confirmed by consumption and reacquisition tests. Figure 9 shows that the Non-devalued animals in both the cholesterol and LNG treatment groups significantly reacquired the nose-poke behavior above and beyond baseline (M = 1.46, SD = 0.38). The Devalued animals did not reacquire the nose-poke behavior (M = 0.17, SD = 0.06). There was a statistically significant difference between devaluation groups in both treatment groups, thus indicating that devaluation was successful ($F_{1, 13}$ = 85.55, $p < .001$, $\eta_p^2 = 0.868$, $\omega^2 = 0.85$, *MSE*= 0.078, 95% CI [0.642, 0.921]; Figure 9).

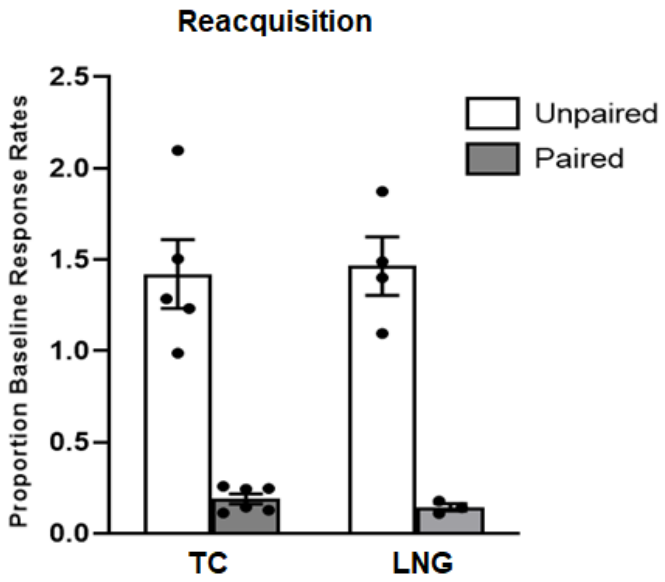

Figure 9. Reacquisition test response rates as a proportion of baseline response rates. Error bars represent SEM; * indicates $p < 0.05$.

Discussion

The results from this experiment indicate that although LNG appears to be enhancing learning during acquisition, it is not accelerating the development of habit; all animals remained sensitive to devaluation and exhibiting goal-directed behavior. This indicates that the exogenous application of a synthetic progestin does not have the same effect as natural progesterone. Prior research has shown that progesterone promotes habitual behavior and seems to be necessary in producing the habit acquisition rate seen in intact females. LNG does not have this same effect, likely because it is not metabolized into the neuroactive metabolite responsible for modulating this behavior acquisition. Experiment I of this thesis shows that progesterone is not accelerating habit acquisition through binding to its classic receptor. This indicates that it must be working through one of its metabolites, which is currently hypothesized to be allopregnanolone. Considering LNG is not metabolized into allopregnanolone, yet does bind to the classic progesterone receptors, it is understandable that it does not generate the same behavioral effects as progesterone.

The results of this experiment indicate that LNG is enhancing the acquisition of the behavior. The animals treated with LNG showed significantly greater behavioral responses on days 2 and 3 of training when compared to their controls. This could potentially be related to the learning system that was relied upon during training. LNG suppresses estrogen and progesterone levels and has a high binding affinity for androgen receptors. There has been a large amount of research showing that male rats and female rats with low estrogen levels prefer striatal response learning strategies, while high levels of estrogen promote the use of hippocampal place strategies (Korol, 2004; Korol et al.,

2004; Korol & Wang, 2018). Thus, the presence of LNG could be enhancing the use of striatal response learning, which could be a faster learning strategy for the instrumental behavior that is being trained. Additionally, LNG could be enhancing DMS activation and improving action-outcome learning. However, it may not have the same effect on the DLS, thus why stimulus-response learning does not exhibit a faster acquisition rate.

The acceleration of action-outcome learning seen in the LNG treated rats was not seen in Experiment 3. An explanation for this could be due to the fact that the Experiment 2 rats were administered fewer reinforcers per VI30 session as compared to the Experiment 3 rats (20 vs. 40 reinforcers, see Chapter 3: Materials and Methods for details). The Experiment 3 acquisition sessions may not have had enough sensitivity to detect differences in learning between the groups. The significant differences in Experiment 2 were observed between days 2 and 3, at 50 and 75 reinforcers delivered. For Experiment 3, the rats received these reinforcer amounts within the same session and differences in learning at these markers were not able to be determined.

A limitation of this experiment is the number of subjects involved ($n_{total} = 18$; $n_{pairedLNG} = 3$). Technically, this experiment is only half completed, and was unable to be finished due to time constraints. The extinction test (critical test for habit) results must be viewed with this in mind, and the number of subjects in this data set must be increased before further extrapolations can be made. However, the acquisition subject numbers are sufficient to draw conclusions from ($n_{LNG} = 7$; $n_{cholesterol} = 11$).

Chapter 3: Investigating the Effect of Levonorgestrel on Habit Behavior in Overtrained Female Rats

Experimental Hypothesis

This experiment tested the impact that LNG has on habit behavior perpetuation in overtrained animals. Rats were given subcutaneous capsules containing LNG, as in experiment II. This experiment examined whether overtrained rats with LNG will exhibit the same habitual behavior as overtrained naturally cycling female rats.

Materials and Methods

Subjects

The subjects of this experiment were 36 gonadally intact, female Long Evans rats obtained from Charles-River Canada. They were implanted with levonorgestrel capsules and maintained at 85% of their free body weight throughout the experiment.

Estrus Cycle Tracking

The rats' estrus cycles were tracked in the same manner as Experiment 2.

Surgery: Levonorgestrel Capsule Implant

All surgical methods were the same as Experiment 2. A total of 15 rats received LNG capsules, and 21 received cholesterol implants. This difference was due to a shortage of LNG to make an equal number of implants.

Training and Testing

As described previously, the training and testing methods were the same for this experiment. During the variable interval 30 second training, rats received 40 reinforcer deliveries over 5 days for a total of 200 reinforcers.

Statistical Analyses

The same statistical analyses as experiment 2, with the same exclusion and correction criteria, were applied to this experiment.

Experimental Timeline

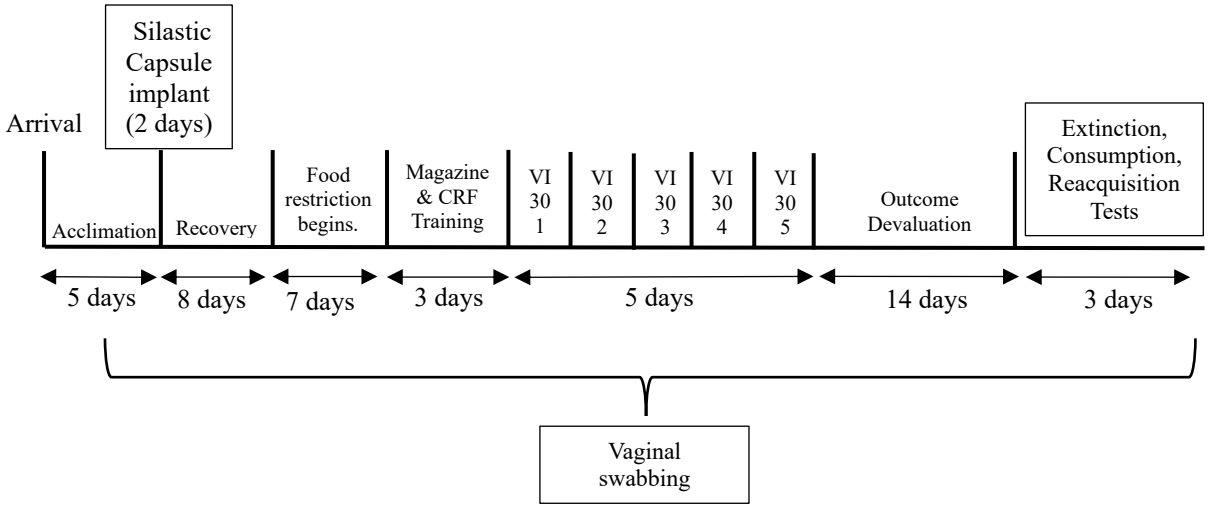

Figure 10. Experiment 3 timeline.

Results

Acquisition

These data indicate that all rats acquired the nose-poking for sucrose reinforcer behavior. The nose-poking behavior consistently increased across the five VI30 training sessions, totaling to 200 reinforcers (Figure 11A). A repeated measures ANOVA confirmed that the main effect of session approaches significance ($F_{3.508,\ 112.3} = 68.692$, $p < .001$, $\eta_p^2 = 0.682$, $MSE = 6.35$, 95% CI [0.576, 0.740]). Acquisition rates between groups were also analyzed. There was no significant difference in acquisition rate between devaluation groups, LiCL paired with reinforcer vs. unpaired ($F_{1,\ 32} = .011$, $p = .917$, $\eta_p^2 = 0.00$, $MSE = 80.3$, 95% CI [0, 0.06]). There was no difference observed between implant groups (LNG vs. cholesterol; Figure 11A), nor a significant interaction effect ($F < 1$). These data suggest that all rats successfully integrated the nose-poke response to sucrose pellet reward.

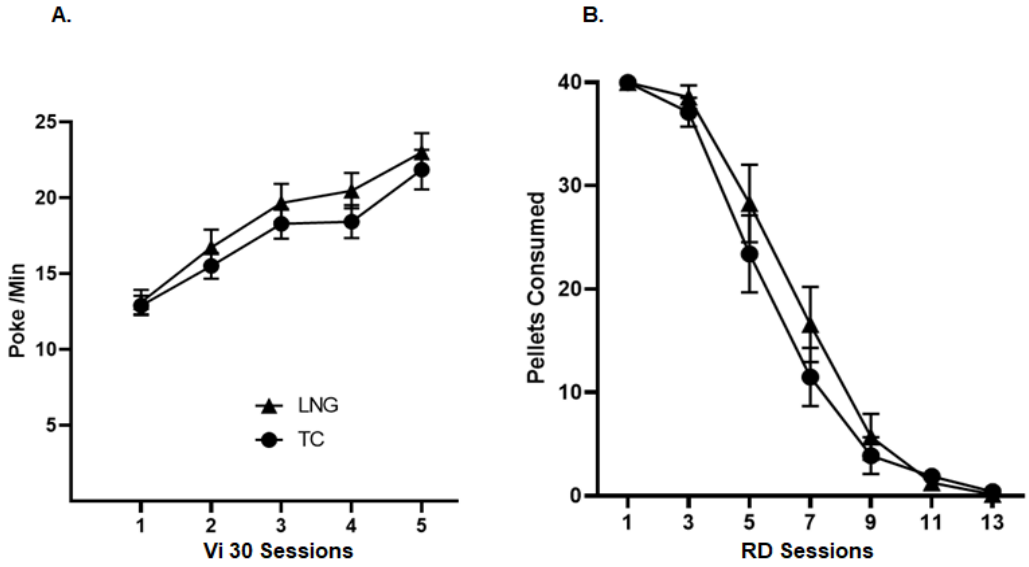

Figure 11. A. Nose-poke acquisition rate across 5 VI(30) sessions. All rats showed successful acquisition. **B.** Devalued group consumption of sucrose pellet across reinforcer devaluation sessions. Devalued rats in both cholesterol and LNG implant treatment groups decreased consumption across the 13 days. Error bars denote SEM.

Outcome Devaluation

All animals in the Devalued group, regardless of implant group, reached zero consumption by the end of reinforcer devaluation (Figure 11B). This indicates that a successful taste aversion was formed to the sucrose pellets. The Non-Devalued group's pellet consumption did not attenuate over reinforcer devaluation, and they did not develop a taste aversion (data not shown). During analysis, Mauchly's test of sphericity was significant (χ^2 (20) = 130.049, $p < .001$) and a Greenhouse-Geisser correction was employed ($\varepsilon = .268$). A repeated measures ANOVA was used to confirm successful devaluation ($F_{1.61, 24.15} = 185.56$, $p < .001$, $\eta_p^2 = 0.925$, $MSE = 94.38$, 95% CI [.843, .95]). There was no significant main effect of implant group (cholesterol vs. LNG) on devaluation rate ($F_{1, 15} = .836$, p=.375, $\eta_p^2 = 0.053$, MSE= 107.7, 95% CI [0, .33]). This suggests that neither cholesterol nor LNG implants have an effect on devaluation.

Test in Extinction

During analysis there were no concerns for outliers, however one rat in the Devalued group failed the consumption test (consumed 6 out of 10 sucrose pellets) and was excluded from further analyses. Analysis of the data indicated a statistically significant effect of devaluation ($F_{1, 34} = 8.686$, $p = .006$, $\eta_p^2 = 0.219$, $\omega^2 = 0.176$, MSE = .010, 95% CI [0.02, 0.411]). The unpaired cholesterol (M = 0.322, SD = 0.113) and unpaired LNG (M = 0.3686, SD = 0.138) groups exhibited higher rates of nose poke responses than the paired cholesterol (M = 0.2612, SD = 0.080) and paired LNG (M = 0.225, SD = 0.046) groups. However, there was no evidence of a statistically significant

effect between treatment groups, cholesterol vs. LNG ($F_{1, 34} = 0.023$, $p = .881$, $\eta_p^2 = 0.001$, $MSE = 0.010$, 95% CI [0.0, 0.08]), nor a significant LNG treatment by devaluation group interaction ($F < 1$). Pairwise comparisons (with a *Bonferroni* correction for the cholesterol rats) was used to analyze response rates within treatment groups. For the cholesterol treated rats there was no significant difference between paired vs. unpaired, indicating that they are responding habitually ($F_{1, 31} = 0.1.918$, $p = .176$, $\eta_p^2 = 0.058$, $Mean_{diff} = 0.061$, 95% CI [-0.029, 0.150]; Figure 12). Evaluation of the Bayes-Factor revealed that there is 1.35 times the indication of the cholesterol capsule treatment group data occurring in support of the null hypothesis – which is habitual behavior ($BF_{01} = 1.35$). In contrast, the LNG treated rats displayed a significant difference between the paired vs. unpaired groups ($F_{1, 31} = 7.152$, $p = .012$, $\eta_p^2 = 0.187$, $\omega^2 = .15$, $Mean_{diff} = 0.144$, 95% CI [0.034, 0.3]; Figure 12). This signifies that they are demonstrating goal-directed responses (Figure 12). Further analysis of the Bayes-Factor showed that there is 3.28 times the indication that the LNG capsule treatment group data will occur in support of the alternative hypothesis – goal directed behavior, with a 0.01% error ($BF_{10} = 3.28$).

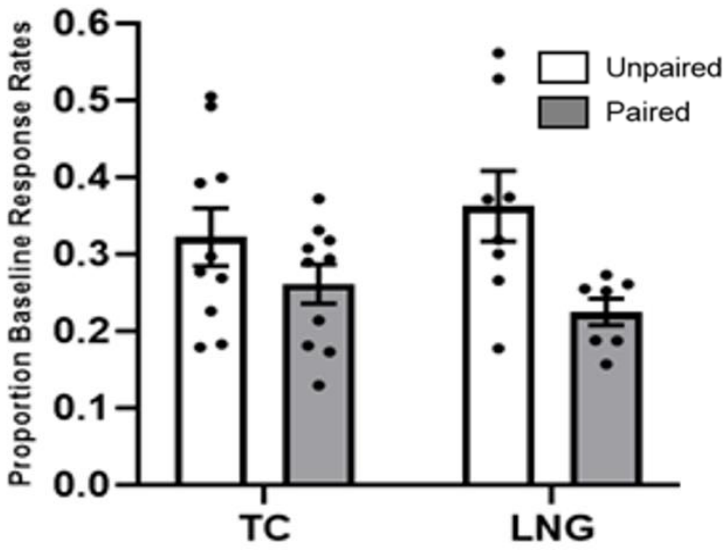

Figure 12. Extinction. Response rates during extinction test as a proportion of baseline response rate. Cholesterol treated rats in the paired condition responded less than the unpaired rats, however this did not approach significance (p = .176). In the LNG treated group, there was a statistically significant difference between rats in the paired and unpaired conditions (p = .012). Error bars denote SEM.

Consumption and Reacquisition Tests

Successful devaluation was confirmed through consumption and reacquisition tests. Figure 13 shows that the Non-devalued animals in the cholesterol (M=1.21, SD = 0.212), and LNG (M = 1.32, SD = 0.23) treatment groups significantly reacquired the nose-poking behavior above and beyond their baseline response rates. The Devalued animals in both cholesterol and LNG groups did not reacquire the behavior, (M = 0.209, SD = 0.067) and (M = 0.23, SD = 0.56) respectively. A statistically significant difference was observed between devaluation groups in both treatment conditions ($F_{1, 34}$ = 352.26, p < .001, η_p^2 = 0.919, ω^2 = 0.91, *MSE* = 0.026, 95% CI [0.843, 0.939]).

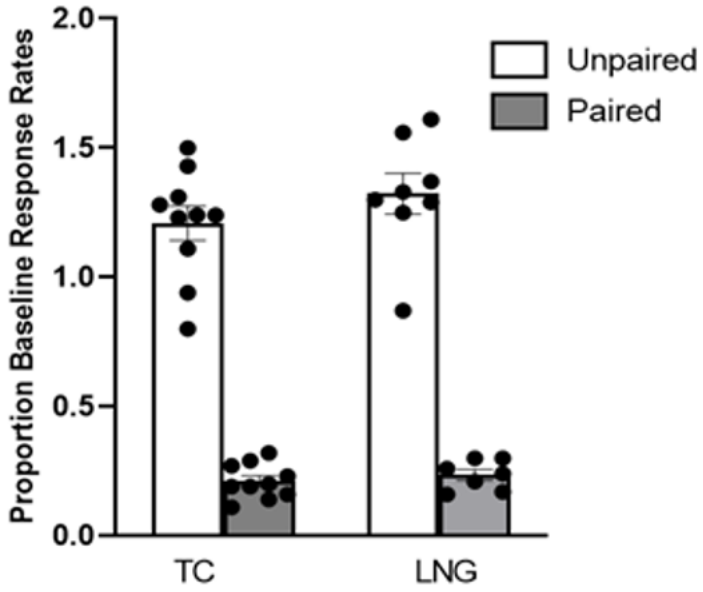

Figure 13. Reacquisition. Response rates during the reacquisition test as a proportion of baseline. All animals in the paired group failed to reacquire nose-poking behavior, regardless of treatment condition. All animals in the unpaired group successfully reacquired nose poking behavior above baseline. Error bars denote SEM.

Discussion

Progesterone is a key hormone in accelerating habit acquisition in female rats, however the results from this experiment indicate that synthetic progestins do not have the same effect, and, in fact, is impairing the development of habit in female rats. The rats treated with LNG did not show signs of habit behavior at 200 reinforcers, where we have consistently seen habit in intact females (or females treated with estrogen and progesterone during acquisition) across a multitude of experimental trials (Schoenberg et al., 2019; Schoenberg, Bremer, et al., 2022). Thus, it is evident that LNG is causing a delay of habit acquisition. This delay in acquisition implies that rats on hormonal birth control, specifically progestin based hormonal birth control, have more behavioral flexibility than naturally cycling female rats.

A potential explanation for this could be due to hormonal birth control's influence on dopamine availability within the striatum. Previous work has shown that increased dopamine sensitization prior to instrumental training delays habit acquisition in female rats (Schoenberg, Evans, et al., 2022). Additionally, functional imaging done in humans has shown that women on hormonal birth control have greater dopamine synthesis and more cognitive flexibility when compared to naturally cycling women (Taylor et al., 2022). These results are consistent with the results from this experiment, and support the conclusions drawn from the data. While there are very few studies investigating the potential of individuals on hormonal birth control having greater cognitive flexibility, these results have provided an interesting launching point for future research to grow from.

An additional explanation for the delay of habit acquisition could be that LNG has a high binding affinity for androgen receptors (Sitruk-Ware, 2006). Synthetic, androgenic progestins (like LNG) could promote physiological impacts and behaviors commonly seen in male animal models (Pletzer & Kerschbaum, 2014). Intact male rats are shown to have extremely delayed habit when compared to intact female rats. Intact males tend to show habit behavior beginning at upwards of 300 reinforcers, while intact females start to show habit at around 120 reinforcers. However, when castrated, male rats show habit behavior at 240 reinforcers. This indicates that male sex hormones are likely involved in delaying habit acquisition. Thus, it is logical to conclude that LNG's effect on the androgen receptor system could be playing a role in the delay of acquisition seen in this experiment. Further research needs to be conducted to solidify this hypothesis,

considering there could be other factors contributing to the delay of habit acquisition seen in males.

Beyond this, progesterone and LNG have been shown to have competing effects on behavior in other aspects of learning and memory. Progesterone is known to promote striatal response learning and memory systems over hippocampal place systems. However, LNG has been shown to promote place memory systems over response memory systems (Lacasse et al., 2023). This is similar to the effects that estrogen has on learning and memory systems. Many different experiments have replicated results that show that high estrogen levels consistently lead to female rats opting for place memory systems over response memory systems (Korol & Wang, 2018; Lacasse et al., 2023). These results are also akin to those seen in male rats. Previous research has also shown that women who have increased androgen receptor sensitivity also opt for place memory systems over response systems (Nowak et al., 2014). There is competing research regarding whether androgenic hormonal birth controls impact tasks that have previously yielded sexually dimorphic results – specifically visuospatial tasks. However, while a general consensus has not been reached, there are many studies that have shown androgenic birth controls improving results on mental rotation tasks when compared to naturally cycling women and women on non-androgenic birth controls (Gurvich et al., 2020; Kuhn & Martini, 2022; Wharton et al., 2008). It is evident that androgen receptor activation is having an effect on cognition and behavior, but there is not a clear picture of where or how these changes are occurring. Future research should investigate these potential cognitive differences that arise as a result of hormonal birth control, and the type of hormonal birth control.

General Discussion

The results from this thesis indicate that LNG has a significant behavioral impact on habit behavior acquisition. The rats treated with LNG exhibited more behavioral flexibility than their naturally cycling counterparts. LNG seems to be delaying habit acquisition, which is the opposite impact that natural progesterone has been shown to have. Intact, naturally cycling female rats only exhibit accelerated habit acquisition with the presence of natural progesterone. However, considering natural progesterone is working through one of its metabolites to accelerate habit acquisition, it is understandable that the results seen with progesterone were not replicated with LNG.

This research attempts to begin closing the gap in literature surrounding women's health and the cognitive/behavioral effects of birth control. Sex as a biological variable has been seriously overlooked due to decades of research that only use male animal models (Shansky & Murphy, 2021). This oversight has resulted in a serious lack of understanding regarding how males and females differ throughout a multitude of areas. One of the excuses for this disparity is that female animals have too many confounding factors (such as cyclic hormones) that would adversely influence results, whereas male models are a "clean slate" that produce results applicable across the sexes (Cahill, 2006). While changing hormone levels can alter behavior across a cycle, the notion that results found in males translate to females has been continuously disproven (Korol & Wang, 2018; Schoenberg et al., 2019). In humans, women and men have been shown to present very differently for psychiatric issues, and many male "warning signs" for physical ailments are not shared in women. These differences, and lack of knowledge surrounding

them, result in grossly underdiagnosing or misdiagnosing a plethora of medical conditions (to name a few: cardiac arrest, strokes, Alzheimer's Disease, attention-hyperactivity disorder, schizophrenia) (Cahill, 2006; Kokras & Dalla, 2014; Shansky & Murphy, 2021). These disparities have been implicated in the higher depression, anxiety, and suicide rates seen in women (Kokras & Dalla, 2014; Shansky, 2009). This serious lack of lack of knowledge surrounding women's health leads to many unnecessary deaths in women that have the potential to be easily prevented. Sex as a biological variable, and understanding how cyclic hormones influence behavior, is an area of neuroscience that must be expanded upon. It is crucial that future research is intentional about increasing knowledge surrounding sex differences if we ever want the medical disparities between men and women to shrink.

In addition to the imbalances present in research regarding men and women, extremely minimal research has been done on the cognitive and behavioral effects of birth control. There has not been a reliable animal model that has been utilized in look into the psychological or physiological impacts of birth control (Hilz, 2022). A majority of the work surrounding birth control has been conducted in human women, and only looks at the contraceptive, hormonal or abortifacient properties. Exploring these aspects of birth control is important for ensuring the safety of the medication, however there are extreme oversight in the literature surrounding the cognitive effects that birth control has been believed/perceived to have. Birth control is prescribed to young individuals for many more reasons than contraception. It is widely used as a medication to "fix" depression, acne, menstrual cramps, irregular periods and premenstrual syndrome. It is clear that hormonal birth control has widespread physiological impacts, far beyond its

contraceptive capabilities (Gurvich et al., 2020). Yet, there is extremely minimal literature surrounding these widespread effects. In fact, from 1960 - 2014, there were only 20-25 studies that had investigated the cognitive impacts of oral contraceptives – the most commonly prescribed birth control, especially in young/adolescent women (Warren et al., 2014). This thesis is seminal work that attempts to fill in one of the many gaps of knowledge surrounding this subject. The lack of research regarding the cognitive impacts of birth control is a huge disservice to the population of people who currently are, have in the past been, or will be prescribed a hormonal contraceptive. As medical professionals continue to prescribe hormonal birth controls, the scientific community must do our part in increasing our understanding of its comprehensive physiological and psychological impacts.

A limitation to this research is that habit development is measured in a very confined operant box; this context is not particularly applicable to the real world. In everyday life, we are presented with multiple stimuli and response options. Often, there are discriminative cues that indicate the habit response is supposed to be conducted. However, in the operant box, there is no distinct cue to respond to and the rats only have one behavior they can execute. Introducing multiple stimuli into the box and giving the rats more choice in their behavioral output would strengthen the results that show they are responding habitually to the nose-poke device.

This thesis opens the door to many unanswered questions regarding the impacts of progesterone and LNG on habit behavior. One of these questions is regarding allopregnanolone's role in accelerating habit acquisition. While allopregnanolone is a metabolite of interest due to its impact on the GABA system, it has not been clearly

shown to affect habit acquisition. Administering cyclic estrogen and allopregnanolone while following the paradigm of Experiment I is a crucial step in further understanding progesterone's role in modulating the early acquisition of habit behavior. Additionally, there are many future directions relating to the impact of LNG on habit behavior. The results from this thesis indicate that LNG is disrupting the acquisition of habit behavior, however the mechanism through which it is doing this is entirely unknown. While a potential hypothesis is its activation of androgen receptors, we do not know if androgen receptor activation is what delays habit acquisition in males. Testosterone is metabolized into dihydrotestosterone (acts on androgen receptors) and estrogen; estrogen has been shown to delay habit acquisition in female rats, and this could be what is accounting for the delay seen in males as well. Further research detailing the mechanism of action through which habit is delayed in male rats is a necessary step for understanding the role that androgen receptors play in habit behavior acquisition rates, and thus if LNG's activation of them is causing a modulatory effect. Another probable hypothesis that should be investigated regarding LNG's effect on habit acquisition is if LNG is modulating the behavior through its impact on other neurotransmitter systems (like the dopaminergic system). Dopamine levels have been shown to impact habit acquisition rates, and hormonal birth control has been shown to have an effect on dopamine synthesis. However, hormonal birth control's impact on the central nervous system is very under researched, and we do not know if it regulates other neurotransmitter levels, or if this potential regulation impacts habit behavior. Additionally, we do not know the rate of habit acquisition in rats treated with LNG. We know that it delays acquisition past the rate consistently seen in intact females, we do not know how many action-outcome

reinforcer pairings are needed for rats treated with LNG to exhibit habitual responding – do they acquire habit at the rate we consistently see in male rats? Do they acquire habit at a rate somewhere between intact females and males? Do they need more action-outcome pairings than males? These are important questions to answer in future experiments regarding habit acquisition rates. Finally, we do not know where LNG is working in the brain – is it modulating behavior at the level of the striatum, or is it acting on a different area? The impact of LNG within the brain is unknown overall, and understanding how and where LNG is producing a behavioral impact is necessary for future research.

References

Allen, R. H., & Goldberg, A. B. (2007). Emergency Contraception: A Clinical Review. *Clinical Obstetrics and Gynecology*, *50*(4). https://journals.lww.com/clinicalobgyn/Fulltext/2007/12000/Emergency_Contraception__A_Clinical_Review.10.aspx

Cahill, L. (2006). Why sex matters for neuroscience. *Nature Reviews Neuroscience*, *7*(6), 477–484. https://doi.org/10.1038/nrn1909

Gemzell-Danielsson, K., Kubba, A., Caetano, C., Faustmann, T., Lukkari-Lax, E., & Heikinheimo, O. (2021). Thirty years of mirena: A story of innovation and change in women's healthcare. *Acta Obstetricia et Gynecologica Scandinavica*, *100*(4), 614–618. https://doi.org/10.1111/aogs.14110

Gurvich, C., Warren, A. M., Worsley, R., Hudaib, A.-R., Thomas, N., & Kulkarni, J. (2020). Effects of Oral Contraceptive Androgenicity on Visuospatial and Social-Emotional Cognition: A Prospective Observational Trial. *Brain Sciences*, *10*(4), 194. https://doi.org/10.3390/brainsci10040194

Hilz, E. N. (2022). Methods and considerations for the use of hormonal contraceptives in rat models of neurobehavior. *Frontiers in Neuroendocrinology*, *66*, 101011. https://doi.org/10.1016/j.yfrne.2022.101011

Hilz, E. N., Olvera, M., Jun, D., Chadha, M., Gillette, R., Monfils, M.-H., Gore, A. C., & Lee, H. J. (2021). *Hormonal contraceptives alter amphetamine place preference and responsivity in the intact female rat* [Preprint]. Animal Behavior and Cognition. https://doi.org/10.1101/2021.07.28.454029

Kokras, N., & Dalla, C. (2014). Sex differences in animal models of psychiatric disorders. *British Journal of Pharmacology*, *171*(20), 4595–4619. PubMed. https://doi.org/10.1111/bph.12710

Korol, D. L. (2004). Role of estrogen in balancing contributions from multiple memory systems. *Neurobiology of Learning and Memory*, *82*(3), 309–323. https://doi.org/10.1016/j.nlm.2004.07.006

Korol, D. L., Malin, E. L., Borden, K. A., Busby, R. A., & Couper-Leo, J. (2004). Shifts in preferred learning strategy across the estrous cycle in female rats. *Hormones and Behavior*, *45*(5), 330–338. https://doi.org/10.1016/j.yhbeh.2004.01.005

Korol, D. L., & Wang, W. (2018). Using a memory systems lens to view the effects of estrogens on cognition: Implications for human health. *Physiology & Behavior*, *187*, 67–78. PubMed. https://doi.org/10.1016/j.physbeh.2017.11.022

Kuhn, H., & Martini, M. (2022). Oral contraceptive androgenicity affects symmetry processing speed in a visuospatial working memory task. *Learning and Motivation*, *79*, 101821. https://doi.org/10.1016/j.lmot.2022.101821

Lacasse, J. M., Boulos, V., Fisher, C., Hamilton, S., Heron, M., Mac Cionnaith, C. E., Peronace, V., Tito, N., & Brake, W. G. (2023). Combined effects of the contraceptive hormones, ethinyl estradiol and levonorgestrel, on the use of place and response memory in gonadally-intact female rats. *Psychoneuroendocrinology*, *147*, 105974. https://doi.org/10.1016/j.psyneuen.2022.105974

Lemus, A. E., Vilchis, F., Damsky, R., Chávez, B. A., García, G. A., Grillasca, I., & Pérez-Palacios, G. (1992). Mechanism of action of levonorgestrel: In vitro metabolism and specific interactions with steroid receptors in target organs. *The Journal of Steroid Biochemistry and Molecular Biology*, *41*(3–8), 881–890. https://doi.org/10.1016/0960-0760(92)90442-L

Liao, P. V., & Dollin, J. (2012). Half a century of the oral contraceptive pill. *Canadian Family Physician*, *58*(12), 757–760.

Ma, G., Song, C., Sun, H., Yang, J., & Leng, X. (2006). A biodegradable levonorgestrel-releasing implant made of PCL/F68 compound as tested in rats and dogs. *Contraception*, *74*(2), 141–147. https://doi.org/10.1016/j.contraception.2006.02.013

McAuley, J. W., Kroboth, P. D., Stiff, D. D., & Reynolds, I. J. (1993). Modulation of [3H]flunitrazepam binding by natural and synthetic progestational agents. *Pharmacology Biochemistry and Behavior*, *45*(1), 77–83. https://doi.org/10.1016/0091-3057(93)90089-C

Nowak, N. T., Diamond, M. P., Land, S. J., & Moffat, S. D. (2014). Contributions of sex, testosterone, and androgen receptor CAG repeat number to virtual Morris water maze performance. *Psychoneuroendocrinology*, *41*, 13–22. https://doi.org/10.1016/j.psyneuen.2013.12.003

Planned Parenthood. (2015). *Birth Control Has Expanded Opportunity for Women—In Economic Advancement, Educational Attainment, and Health Outcomes. [Fact Sheet]* (pp. 1–4). https://www.plannedparenthood.org/files/1614/3275/8659/BC_factsheet_may2015_updated_1.pdf

Pletzer, B. A., & Kerschbaum, H. H. (2014). 50 years of hormonal contraceptionâ€"time to find out, what it does to our brain. *Frontiers in Neuroscience*, *8*. https://doi.org/10.3389/fnins.2014.00256

Pridjian, G., Schmit, V., & Schreiber, J. (1987). Medroxyprogesterone acetate: Receptor binding and correlated effects on steroidogenesis in rat granulosa cells. *Journal of Steroid Biochemistry*, *26*(3), 313–319. https://doi.org/10.1016/0022-4731(87)90095-1

Schindler, A. E., Campagnoli, C., Druckmann, R., Huber, J., Pasqualini, J. R., Schweppe, K. W., & Thijssen, J. H. H. (2008). Reprint of Classification and pharmacology of progestins. *Maturitas*, *61*(1–2), 171–180. https://doi.org/10.1016/j.maturitas.2008.11.013

Schoenberg, H. L., Bremer, G. P., Carasi-Schwartz, F., VonDoepp, S., Arntsen, C., Anacker, A. M. J., & Toufexis, D. J. (2022). Cyclic estrogen and progesterone during instrumental acquisition contributes to habit formation in female rats. *Hormones and Behavior*, *142*, 105172. https://doi.org/10.1016/j.yhbeh.2022.105172

Schoenberg, H. L., Evans, C., Bausch, L., Wootton, A., Kirshenbaum, A., & Toufexis, D. J. (2022). Dopamine sensitization by methamphetamine treatment prior to instrumental training delays the transition into habit in female rats. *Behavioural Brain Research*, *418*, 113636. https://doi.org/10.1016/j.bbr.2021.113636

Schoenberg, H., Sola, E., Seyller, E., Kelberman, M., & Toufexis, D. (2019). Female Rats Express Habitual Behavior Earlier in Operant Training Than Males. *Behavioral Neuroscience*, *133*. https://doi.org/10.1037/bne0000282

Schumacher, M., Mattern, C., Ghoumari, A., Oudinet, J. P., Liere, P., Labombarda, F., Sitruk-Ware, R., De Nicola, A. F., & Guennoun, R. (2014). Revisiting the roles of progesterone and allopregnanolone in the nervous system: Resurgence of the progesterone receptors. *Allopregnanolone: State of the Art*, *113*, 6–39. https://doi.org/10.1016/j.pneurobio.2013.09.004

Shansky, R. M. (2009). Estrogen, stress and the brain: Progress toward unraveling gender discrepancies in major depressive disorder. *Expert Review of Neurotherapeutics*, *9*(7), 967–973. https://doi.org/10.1586/ern.09.46

Shansky, R. M., & Murphy, A. Z. (2021). Considering sex as a biological variable will require a global shift in science culture. *Nature Neuroscience*, *24*(4), 457–464. https://doi.org/10.1038/s41593-021-00806-8

Sitruk-Ware, R. (2006). New progestagens for contraceptive use. *Human Reproduction Update*, *12*(2), 169–178. https://doi.org/10.1093/humupd/dmi046

Stovall, D. W., Aqua, K., Römer, T., Donders, G., Sørdal, T., Hauck, B., Llata, E. S. de la, Kallner, H. K., Salomon, J., Zvolanek, M., Frenz, A.-K., Böhnke, T., & Bauerfeind, A. (2021). Satisfaction and continuation with LNG-IUS 12: Findings from the real-world kyleena ® satisfaction study. *The European Journal of Contraception & Reproductive Health Care*, *26*(6), 462–472. https://doi.org/10.1080/13625187.2021.1975268

Taylor, C. M., Furman, D. J., Berry, A. S., White, R. L., Jagust, W. J., D'Esposito, M., & Jacobs, E. G. (2022). Striatal dopamine synthesis and cognitive flexibility differ between hormonal contraceptive users and non-users. *BioRxiv*, 2022.10.20.513082. https://doi.org/10.1101/2022.10.20.513082

Vashchinkina, E., Panhelainen, A., Aitta-aho, T., & Korpi, E. R. (2014). GABAA receptor drugs and neuronal plasticity in reward and aversion: Focus on the ventral tegmental area. *Frontiers in Pharmacology*, *5*. https://www.frontiersin.org/article/10.3389/fphar.2014.00256

Warren, A. M., Gurvich, C., Worsley, R., & Kulkarni, J. (2014). A systematic review of the impact of oral contraceptives on cognition. *Contraception*, *90*(2), 111–116. https://doi.org/10.1016/j.contraception.2014.03.015

Whaley, N. S., & Burke, A. E. (2015). Intrauterine Contraception. *Women's Health*, *11*(6), 759–767. https://doi.org/10.2217/whe.15.77

Wharton, W., Hirshman, E., Merritt, P., Doyle, L., Paris, S., & Gleason, C. (2008). Oral contraceptives and androgenicity: Influences on visuospatial task performance in younger individuals. *Experimental and Clinical Psychopharmacology*, *16*(2), 156–164. https://doi.org/10.1037/1064-1297.16.2.156