UiT
THE ARCTIC UNIVERSITY OF NORWAY

Department of Psychology

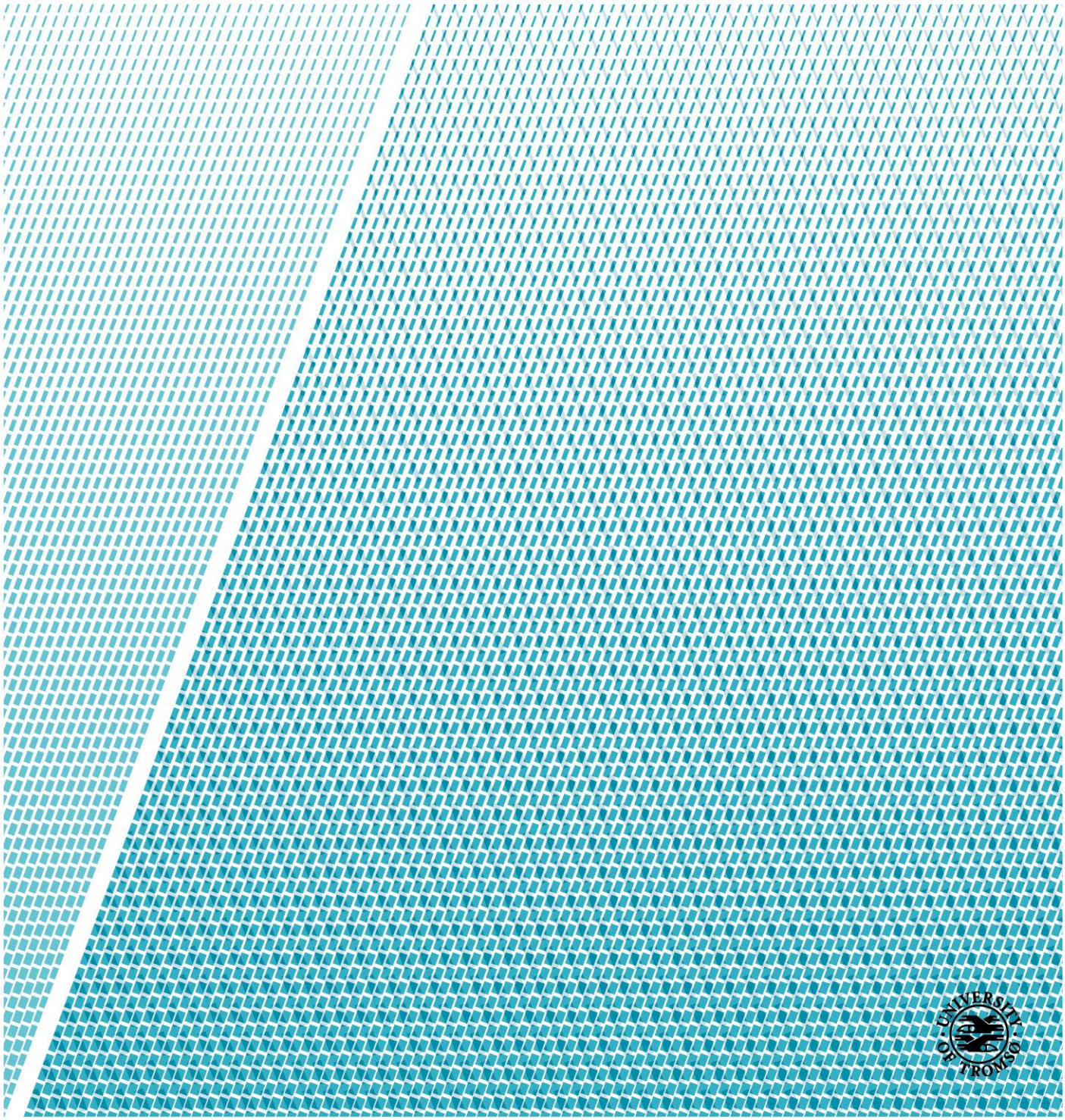
Can Perinatal SSRI Exposure Affect Sexual Behavior Later in Life?

Early Developmental Fluoxetine Exposed Female Rats Observed in a Seminatural Environment

—

Jan Hegstad

Master's thesis in psychology - May 2019



Can Perinatal SSRI Exposure Affect Sexual Behavior Later in Life?

Early Developmental Fluoxetine Exposed Female Rats

Observed in a Seminatural Environment

Jan Hegstad

Supervisor: Eelke Snoeren

PSY-3900 Master Thesis in Psychology

UiT The Artic University of Norway

Tromsø, May 2019

Preface

When I was fishing for a project during my bachelor I was in contact with associate professor Eelke Snoeren, who at that time recommended me to come back for a master project. So, when I returned as a master student she offered me a project where I would look at the behavior of rats that were exposed to antidepressants during early development. This was totally new to me, and I figured I would learn a lot if I took this project. However, since there are no available courses in biology at a master's level to support me, I expected that the road ahead could be steep.

Since the experiment involving the rats had already been run, my job was to score the different behaviors during one specific day. I received training in how the software worked and how to identify the different behaviors, and after some follow-up controls from Eelke, I did all the scoring. The partitioning of the entire observational day into the bouts, compiling the data in Excel, making the graphs and analyzing the data is done by me, with Eelke providing the instructions on how to set it up.

The text is written by me, whereas Eelke provided input on structure (A before B) and sometimes lent a hand on retrieving some of the older research articles (like the Ball, 1937). The interpretation of the data is done by me, although Eelke has been involved in allowing me to structure my thoughts along the way. In summary, it has been an amazing experience where I've learned a lot, although the road proved to be long indeed.

I would like to thank:

My fellow inhabitants of office 5.265 for their contributions on my life as a student.

The members of the behavioral and translational neuroscience research group for their hearty inclusion.

My supervisor Eelke Snoeren, this thesis would not exist without you.

Friends and family that have allowed me to immerse myself in this project.

Jan Hegstad, student

Eelke Snoeren, supervisor

Sammendrag

Forskning på området seksualadferd har i senere tid tatt seg opp på basis av rapporterte negative symptomer av antidepressive medikamenter av typen selektive serotonin reopptaks hemmer (SSRIs). Bruken av SSRIs, og særlig under graviditeten, kan påvirke utviklingen av barnets hjerne, siden medikamentet krysser morkaken (placenta) og i tillegg er til stede i brystmelk. Perinatal eksponering av SSRI gir en økning av serotonin i hjernen i faser av utvikling der stoffet har nevrotrofisk virkning. Tidligere forskning har vist at eksponering i voksen alder har gitt en reduksjon i seksualadferd hos kvinner, hva effekten av en perinatal eksponering vil være vet vi derimot lite om. Studien vår utforsker derfor effekten av perinatal SSRI eksponering og påvirkningen på voksne rotters når de er testet i et seminaturelt miljø.

Rottemødre fikk enten 10 mg/kg fluoxetine (FLX) eller placebo (vehicle, Ctrl) gjennom en sonde i munnen, fra dagen de ble gravide (G0) til avvenningsdag 21 (postnatal dag 21). Testingen av avkommet skjedde i grupper på åtte dyr (fire hunndyr og fire hanndyr) i et seminaturelt miljø. Hunndyrene var ooforektomiser før de ankom innhegningen og ble senere tilført hormoner for å bli seksuelt aktive. Atferden deres ble skåret og senere analysert fra tidspunktet der de først viste en lordosis til den siste observerbare lordosis (hele brunst perioden), og for hver seksuelt aktive periode (en periode ble avsluttet når det ikke forekom en ny lordosis innen en time) innad i brunstperioden.

Det finnes ikke støtte i data som sier at perinatal fluoxetine eksponering påvirker seksualadferd i voksen alder. Videre viser vår data at lengen på brunstperioden og de seksuelle episodene er helt like for Flx hunndyr som for Ctrl hunndyrene. Selv med fokus på den episoden med mest seksuell adferd i, så finner vi ingen forskjell. Med bakgrunn i denne studien konkluderer vi med at perinatal SSRI eksponering ikke påvirker seksualadferd i hunndyr.

Nøkkelord: Fluoxetine, perinatal, seksualadferd, hunnrotter, seminaturelt miljø

Abstract

Renewed interests in sexual behavior research stems from human reports of negative symptoms of selective serotonin reuptake inhibitor (SSRI). The use of SSRIs, during pregnancy might impact the developing child, due to the drug's ability to cross the placenta and also be present in breastmilk. Perinatal SSRI exposure thus elevates serotonin levels in the developing brain, during phases where serotonin acts as a neurotrophic factor. Previous literature reported that SSRIs given to adults affect female sexual behavior in a negative way, in contrast little is known regarding the effects of perinatal SSRI exposure on offspring. Our study investigates perinatal fluoxetine exposure in rats and the effects on female sexual behavior during the length of the behavioral oestrous cycle, housed in a seminatural environment.

Mothers received daily oral gavage of 10 mg/kg fluoxetine (FLX) or vehicle (Ctrl) from gestational day 0 until postnatal day 21. Testing of adult offspring consisted of cohorts of eight rats (four females and four males) in a seminatural environment. All females were ovariectomized before entering and then hormonally primed on the 7th day in the environment to induce sexual receptivity. Female sexual behavior was scored and analyzed from the first and including the last lordosis (behavioral oestrous period), and within each copulatory bout (a bout ended when there was no lordosis within the next hour) during the behavioral estrus.

None of the data indicate that perinatal fluoxetine exposure affects female sexual behavior at adulthood. We further found that the behavioral estrus of Flx females had the same length and pattern as Ctrl females. When the behavioral oestrous was divided in several copulatory bouts, with a focus on the most active one, no differences in behavior was found. Thus, we conclude that perinatal SSRI exposure does not affect female sexual behavior.

Keywords: Fluoxetine, perinatal, sexual behavior, female rats, seminatural environment

Despite the ever-growing body of research involving animals, there are, for instance, elements regarding the sexual behavior of rats that still remains underexplored especially on the topics of sexual competition and partner choice. An observer might notice the biggest behavioral changes when the female encounters a male, if for instance the female is in *dioestrous* (not sexually receptive) she will often respond to an approaching male with rejection (Chu & Agmo, 2014). The rejection is shown in a stereotypical set of motor patterns, for example with kicking or facing off the male rat. On the other hand, if the female happens to be in *oestrus* she will probably respond to the presence of the male with different motor patterns such as ear wiggling, darting (proceptive) or lordosis (receptive).

The presence of hormones is required for the animal to display certain behaviors, an example of this is the lordosis response (arching of the back, elevation of the rump, swinging the tail to one side, and extension of the neck) in females. In intact animals the circulation of gonadal hormones not only determines sexual behavior, the hormones also modify general arousal (Pfaff et al., 2002), fear and anxiety (Frye & Walf, 2004), social recognition (Hlinak, 1993), object memory (Walf et al., 2006) and aggression (Albert et al., 1992). At the present time we cannot rule out the possibility of additional behavioral actions being affected by hormones (Chu & Agmo, 2014).

In a naturally cycling rat the hormones that regulates sexual receptivity are estrogen (E) and progesterone (P), and the brain sites and mechanisms of those hormones are reasonably well known (see Agmo, 2011; Pfaff, 1999, for reviews). Roughly explained, estrogen is involved in the lordosis response, while progesterone is involved in proceptive behaviors (Uphouse, 2000). This is somewhat different in the case where the female rat has undergone removal of the ovaries (ovariectomy, OVX). When dealing with OVX females, the order in which the hormones are administered is important. If P is injected before E, it reduces sexual behavior, and if P is injected without the presence of E very little happens.

Therefore, estrogen is administered at least 24 hours and preferably 48 or 72 hours before the dose of progesterone is given (Pfaff, 1999, p. 57). The hormonal dosages is also important, if for instance the dose of estrogen is too low, the female will show a reduction in lordosis behavior and the length of sexual receptivity is shortened (Uphouse, 2000).

What behaviors are then involved in the term sexual behavior? Usually the behavior is divided into three phases: *precopulatory phase*, *copulatory phase* and *executive phase* (Snoeren, Veening, Olivier, & Oosting, 2014a). In female rats the precopulatory phase involves approach and investigating the male by sniffing i.e. the anogenital regions. Proceptive behaviors are such as: *hopping* (short jumps with all four legs off the ground), *darting* (short and sudden runaway moments, in which she presents her body to the male) and *ear wiggling* (lateral shaking of the head making the ears appear to wiggle). By displaying such behaviors, she aims to draw the attention of a male rat, and often he will give chase to the female and attempt to *mount* her, thus they transition into the copulatory phase. The male will straddle her from behind, thrusting his hips attempting to locate her vagina with his penis. The stimulation provided by the male will result in the female showing a *lordosis*, if however, the female does not show this behavior, the male will struggle with *intromission* and also *ejaculation*. A successful insertion of the male's penis will result in a deeper thrusting movement from the male, followed by a dismount (Heijkoop, Huijgens, & Snoeren, 2017). This behavior is recognized as an *intromission.* As a response to mounts and intromissions the female will arch her back and swing her tail to the side for better vaginal entry. This receptive behavior is known as a *lordosis* (Fig 1, bottom left). The above-mentioned behaviors tend to proceed in rapid succession only to be intermitted by self-grooming, rest and *pacing* by the female (run-away behavior). The male will at some point reach an ejaculation where he often will make a small jump backwards and raising his forepaws in the process (Figure 1, bottom right). Ejaculation is considered as the end point for male sexual behavior, where the male will often remain

inactive for about 5 minutes afterwards (Heijkoop et al., 2017). On the other hand, females do not seem to have the same cool down in their mating scheme and can therefore resume copulation with a different male much faster.

By contrast, in humans, we find that the variety of postures associated with sexual behavior may seem unlimited. Thus when researchers are investigating sexual behavior, such studies often make use of animals whose behavior repertoire involves fewer complicated movements (although see Agmo, 2011, p. xvi). Sexual behavior in rats consist to a larger degree of stereotypical behaviors, making rats a preferred model for investigating, with translational purpose, sexual behavior (Heijkoop et al., 2017; Uphouse, 2014).

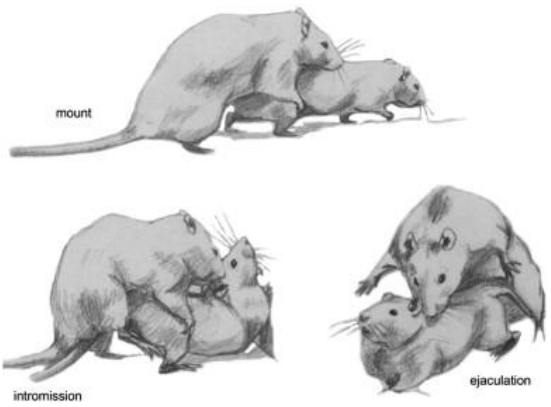


Figure 1. *Sexual behavior of male rats: mount, intromission, and ejaculation are shown in the drawings by P.J.A. Timmermans* (Snoeren, Veening, Olivier, & Oosting, 2014b).

Not only are the behavioral repertoire different from humans to for instance rats, as experienced by most human females, they have sex regardless of their internal hormonal state. Whereas the female rat most likely will reject an approaching male based on her endocrine state, a human female has the choice of accepting an approaching male or she can choose to reject him. Such behaviors are harder to model in animals, especially in rats where if you have a receptive female and a male, the behaviors they will display are predominantly sexual behaviors. However, as noted in Snoeren et al. (2011), female rats tend to segregate into three groups when tested repeatedly, hypoactive (underperforming), normal or hyperactive

(overperforming) in their sexual behavior. Thus, it seems that there are additional mechanisms involved in female sexual behavior besides the endocrine state.

One of the systems that was hypothesized to be involved in sexual behavior was the serotonin (5-HT) system. Over the years there is written several excellent reviews on the topic of 5-HT involvement in sexual behavior, for a more in-dept desctiption of 5-HT involvement see Mendelson, (1992); B. Olivier et al. (2010); Snoeren et al. (2014a); Uphouse (2014). Some of the reports claim that in adults, heightened extracellular levels of 5-HT in the brain are reported to decrease sexual behaviors. The most common way to increase 5-HT levels in adults are through drugs called selective serotonin re-reuptake inhibitors (SSRIs), a drug that is predominantly prescribed is treatments of mood disorders (Pawluski, Li, & Lonstein, 2019). SSRIs target the 5-HT transporter, disabling its ability to recycle released 5-HT from the synaptic cleft, thus elevating the amount of serotonin available to the post-synaptic neuron.

The reported effects of SSRIs are not showing a unison picture. Some researchers found that chronic SSRI exposure in adult rats decreased paracopulatory and lordosis behavior, in other cases it facilitated such behaviors (Mendelson, 1992). Several different receptors were discovered in studies on 5-HT, and it was, for instance, shown that if the 5-HT_{1A} receptor is activated then lordosis behavior will be reduced (Snoeren et al., 2014a). On the other hand, activation of the 5-HT_{2A} and/or 5-HT_{2C} was shown to be involved in the facilitation of female sexual behavior.

Serotonin is, however, not only involved in sexual behavior as a neurotransmitter. During the early stages of fetal development, 5-HT act as a neurotrophic factor and is involved the control of proliferation, differentiation, migration, cell death, synaptogenesis and dendritic pruning (J. D. Olivier, Blom, Arentsen, & Homberg, 2011). On the presumption that brain plasticity is higher in newborns, children and adolescents, researchers began to speculate about possible consequences when pregnant mothers took SSRIs. As noted earlier, SSRIs are often

prescribed to elevate depressive symptoms, and there is a presumed safety associated with taking SSRIs during pregnancy. Women have an increased risk to suffer from depression during pregnancy, and if the depression is not treated, the depression itself can have detrimental effects on the child. As such, treatment of depression with SSRIs would be a logic consequence. -However, SSRIs are shown to cross the placenta and the drug is also present in breastmilk, resulting in heightened 5-HT levels in the fetus and newborn (J. D. Olivier, Valles, et al., 2011).

Where previous literature on SSRI exposure mainly looked at chronic exposure and its effect on adults, there are fewer studies on postnatal exposure and the effects later in life. If we narrow in our interests even further and search for perinatal exposure SSRI exposure and the effects on the exposed sexual behavior once adults, there is even less knowledge to be found, especially when it concerns the effects on sexual behavior later in life. Currently, there is only one study on postnatal *fluoxetine* (FLX, an SSRI) exposure. They reported an increase in females sexual behavior after early life FLX exposure once they became adult rats (Rayen, Steinbusch, Charlier, & Pawluski, 2014). In their study Rayen et al. (2014) administered fluoxetine to the mothers immediately starting their postpartum period. Thus, the newborn pups were exposed to FLX from their birth until they were weaned. Once the females reached adulthood they were submitted to a 45-minute copulation test, where they assessed the females' paracopulatory and lordosis behaviors. Results from the test showed an increase in both behaviors compared to the control animals. The study by Rayen et al. (2014) complies nicely with the “SSRI paradox”, which claims that developmental SSRIs exposure results in opposite effects than chronic exposure during adulthood.

The aim of the preset study is therefore to help close the knowledge gap on perinatal SSRI exposure to fetuses and the potential effects of that exposure once the animals are adults. There is however one critical difference in our experimental design compared to the study by Rayen et al. (2014); we will make use of a seminatural environment as testing grounds for the

rats' behaviors. Such a setup has been previously used by McClintlock and Adler (1978) and later by Chu and Agmo (2014) to study female sexual behavior in greater details compared to standardized copulation tests. By not limiting the animals to shorter tests in confined spaces the rats are free to express whatever behavior they deem fit. In addition, we are able to investigate the complete behavioral oestrous period from the start when the female becomes receptive until the end. Finally, since the rats live in groups, we are also able to explore the element of mate choice in the seminatural environment. As a consequence, our methodological aspect allows for a deeper understanding regarding the effects of perinatal SSRI exposure on female sexual behavior in a more natural setting.

Based on the results from Rayen et al. (2014) we expect to also see an increase in female rats' sexual behaviors. Since our design features OVX rats which needs to be primed with hormones for them to be receptive, we do not expect a difference in the latency to start copulating between the vehicle group (Ctrl) and the fluoxetine (Flx) exposed group. The expected increase in sexual behaviors shown as higher numbers of lordosis behaviors and time spent on for instance paracopulatory behaviors in FLX exposed females, could be caused by the fact that the FLX exposed females might have a longer, and thereby less efficient behavioral oestrous period than Ctrl females.

2. Materials and methods

2.1 Subjects

Wistar rats (N = 20, 10 females and 10 males) were purchased from Charles River (Sulzfeld, Germany) for breeding purpose. They become the dams and fathers of the offspring. Housing conditions were same sex pairs in Makrolon® IV cages for parents and future offspring, room temperature was kept at 21 ± 1 °C, humidity at 55 ± 10% on a 12:12 h light/dark cycle (lights on 11:00 h). Commercial rat pellets (Standard chow from SDS, Special Diet Services) along with tap water were provided ad libitum, and nesting material was also present.

Animal treatment and experiments complied with European Union council directive 2010/63/EU. The protocol was approved by the National Animal Research Authority in Norway.

2.2 Breeding and Antidepressant Treatment

To determine female receptivity of the future dams, all females spent 5 minutes daily with a male. They were considered receptive when they displayed a lordosis in response to a mount. When receptive, the females were placed with a male for approximately 24 hours (Gestational day 0). During these hours, each female-male couple was housed in a Makrolon® IV cage. After 24 hours, male and female returned to their original home-cages (with their previous same-sex partner). On gestational day 14, the females were single housed in Makrolon® IV cages with access to nesting material.

From gestational day 1 (G1) until postnatal day 21 (PND21), females were administered daily with either 10 mg/kg fluoxetine (Apotekproduksjon, Oslo, Norway) or a vehicle (Methylcellulose 1%, (Sigma, St. Louis, MO, USA)) using gavage with a stainless steel feeding needle (total of 6 weeks). The fluoxetine drug was prepared from fluoxetine tablets (for human usage), which were pulverized and dissolved in sterile water (2mg/ml) and fused orally at a volume of 5ml/kg. As control condition, methylcellulose powder, the non-active filling of fluoxetine tablets, was dissolved in sterile water to create a 1% solution and administered at a volume of 5ml/kg as well. The amount of vehicle/fluoxetine given was adjusted upon the weight of the females who were weighed every three days. The dose of fluoxetine was based on comparison to human situations (J. D. Olivier, Valles, et al., 2011). When delivery was imminent, dams were checked at 09:00 h and again at 15:00 h.

2.3 Offspring Housing Conditions Before the Seminatural Environment

At no point were the litters culled. Weaning of pups was on post-natal day 21, and from that point they were housed in groups of two or three same sex littermates in Makrolon® IV. Cages were cleaned twice a week, this was the only human interaction the pups experienced prior to being introduced to the seminatural environment (at 13 weeks of age). Females underwent ovariectomy at 11 weeks of age (see 2.4). Ears were punched for individual recognition at the moment they were weaned.

2.4 Ovariectomy Surgery

Ovariectomy of females enabled manipulation of their estrous cycle with hormone injections. Copulation behavior is often dominant to other behaviors, and thus the purpose of the hormone injection was to limit copulation behavior to the last day of the observation period.

Females were given isoflurane anesthesia and were placed on their ventral surface. In addition, buprenorphine (.05 mg/kg) and Carprofen (5mg/kg) were given subcutaneously in the upper neck region of the animal before surgery. Ovariectomy was preceded by an 1-2 cm longitudinal midline dorsal skin incision at the lower back of the animal. Muscle incisions were made bilaterally and the peritoneal cavity was accessed. The ovary was located, the connection between the fallopian tube and the uterine horn ligated, and the ovary was extirpated. Muscle incisions were sutured and a wound clip was placed for skin closure. Animals were given Carprofen (5mg/kg subcutaneously) 24 and 48 hours after surgery. Female offspring were single housed for 3 days during recovery before returning to their homecage.

2.5 Design

The seminatural environment had one cohort of rats at the time, for a total of four cohorts, videotaped for behavioral observations. Each cohort consisted of four males and four females (offspring animals), balanced with two offspring from control mothers and two from fluoxetine treated mothers for both sexes. For data analysis we thus had eight animals per

observation group (mixed in four groups); eight females and eight males that were exposed to fluoxetine during development (Flx-females and Flx-males, respectively), and eight females and eight males that were exposed to vehicle during development (Ctrl-females and Ctrl-males, respectively).

Cohorts were created by selecting pups from different litters, with a combination of pups so that animals of the same sex were not littermates. However, within a cohort, almost every animal had 0 - 1 sibling from the opposite sex due to a limited amount of litters available. All littermates were housed for three months in different home cages after weaning. Offspring were otherwise unfamiliar to each other and sexually naive.

2.6 Procedure

The day preceding introduction to the seminatural environment every animal was marked. This was done by shaving their backs in different areas and marking their tails with different stripes (Appendix A).

The offspring was placed in the seminatural environment for 8 days (day 0 – day 8). An overview of the whole procedure from the beginning of antidepressant treatment until the end of testing of the offspring is given in Figure 2. Animals entered the seminatural environment on the first day (Day 0) at 10:00 h introducing first the females, then the males in the open field section of the seminatural environment. On day 8, the animals were removed at 10:00 h, marking the end of the experiment. For the duration of one experiment, the seminatural environment was not cleaned, this procedure was done in the 48 hour interval between experiments when it was also disinfected.

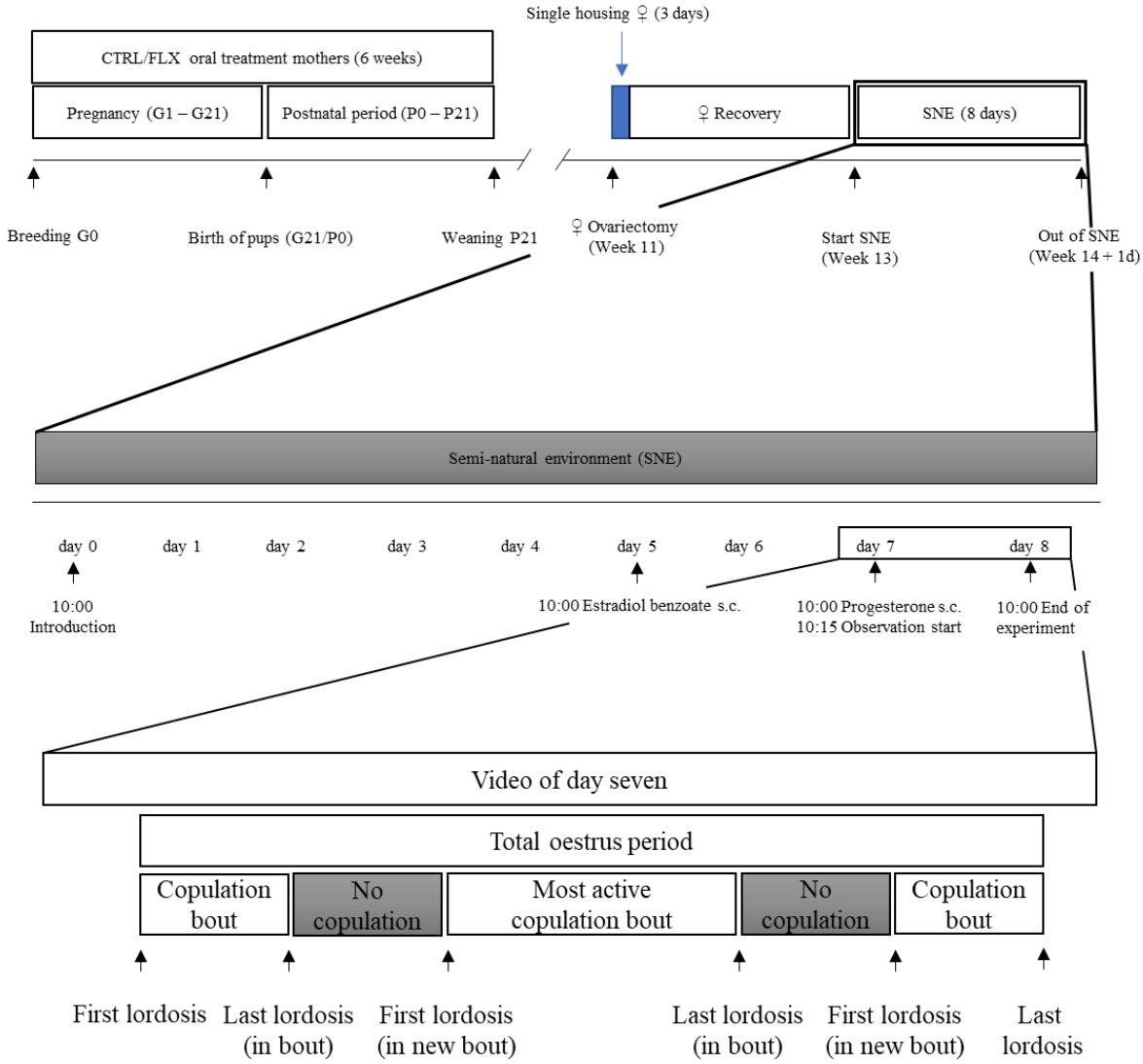


Figure 2. Schematic overview of all experimental procedures.

2.7 Seminatural Environment

The seminatural environment (Figure 3) was designed to mimic the environment of wild rats. A similar setup has been used by McClintock and Adler in their studies (e.g., McClintock & Adler, 1978; McClintock et al., 1982). For a detailed description of the apparatus see Chu and Agmo (2014), for the technical details concerning this experiment see Houwing, Heijkoop, Olivier, and Snoeren (2019). Briefly, the seminatural environment measured 2.4 x 2.1 x 0.75 meters, consisted of a burrow system (tunnels and nest boxes) and an open field area which were connected by four 8 x 8 cm openings. A black curtain separated the two arenas, enabling the burrow section to remain dark while the open area ran simulated

day-night cycles in reverse. From 10:30 h to 11:00 h the light gradually decreased to approximately 1 lux at floor level, this was kept constant until 22:15 h when the light increased in increments until 180 lux at 22:45 h.

The entire floor was covered in aspen wood shavings, 6 squares of nesting material was put in the nest boxes, 12 wood sticks and 3 red plastic shelter huts were placed in the open area along with approximately 2 kg of food. Water was provided freely by four bottles mounted in one of the corners in the open area.

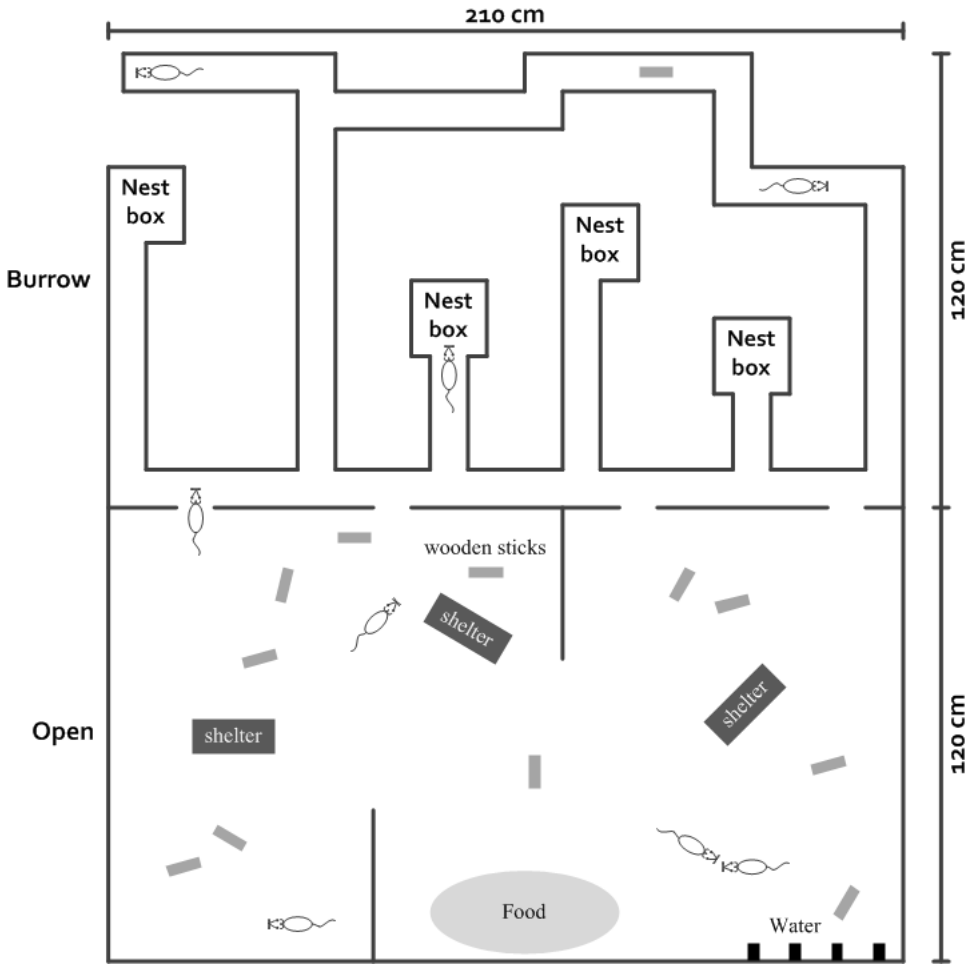


Figure 3. Overview of the seminatural environment.

2.8 Hormone Treatment

On day 5 and 7, females were for a short duration removed from the apparatus in order to receive a subcutaneous hormone injection. This was done to induce sexual receptivity on day 7. The ovariectomized females received 18 µg/kg estradiol benzoate (Sigma, St Louis,

MO, USA) on day 5, and 1 mg of progesterone (Sigma, St Louis, MO, USA) on day 7, both doses were dissolved in peanut oil (Apoteksproduksjon, Oslo, Norway) and injected in a volume of 1 ml/kg. Injections were given at 10:00 h and females were placed back at the same place into the burrow part of the seminatural environment. Since the males did not receive any hormone injections, they were left undisturbed in the seminatural environment. The doses and timing of the estradiol and progesterone injections were based on previous research showing that it produces maximal receptivity and high intensity of female reproductive behavior (see Spiteri et al., 2010).

2.9 Behavioral Analysis

The frequency and/or duration of a wide variety of behaviors was scored by the author, blind for the treatment of the animals, using the Observer XT version 12.5 (Noldus, Wageningen, The Netherlands) software. Videos of day seven were examined from the start of that day but no behaviors were registered before a female displayed a lordosis. This marked the beginning of "*behavioral oestrus*" and was also the start of one "*copulatory bout*" (Figure 2). I then scored the behavior of each copulatory bout, which consisted of the time between the first and last lordosis. The last lordosis was defined as the lordosis which no other lordosis occurs within 1 hour. A new "copulatory bout" would be initiated by a new lordosis starting after the previous "cut-off" point of 60 minutes, until again no lordosis was observed for 60 minutes. Thus a "behavioral oestrus" could in theory contain one or multiple "copulatory bouts" where all ended with their respective lordoses.

Behaviors listed in Table 1 were scored from the females' perspective, male behavior was ignored except for when they interacted with a female (e.g. mounted by or female sniffing male). Behaviors that occurred between the bouts was also scored, however since they fall outside the defined periods of bouts, as a consequence these behaviors are excluded in the following analyses.

Table 1

Description of the registered behavior

Sexual behavior	
Paracopulatory behaviors	Approach to a male followed by runaway, often associated with hops, darts, ear wiggling
Lordosis	Receptive behavior with a hollow back and deflects tail to one side
Mounts received	Mounting on the rump of another rat from behind with pelvic thrusting
Intromissions received	Mounts including penile insertion
Ejaculations received	Penile insertion lasts longer than at intromission and is associated with rhythmic abdominal contractions
Social behaviors	
Grooming others	Self-explanatory.
Sniffing others	Sniffing any part of the body, except the anogenital region. Performed by males as well as females.
Anogenital sniffing	Self-explanatory. Performed by males as well as females.
Conflict behavior	
Boxing/wrestling	One or both animals are pushing, pawing and grabbing at each other using their forepaws
Rejection	Kicking other rats, either on all four or belly up. Only female rejections were recorded
Nose off	Facing another rat either standing on four legs or while rearing. Performed by males as well as females.
Any other behavior	Non-targeted behavior e.g. sleeping, walking, sniffing objects or walls.

In addition to determine the frequency and/or duration of the behaviors listed in Table 1, the lordosis quotient (LQ) was calculated by dividing the number of lordoses displayed by the number of mounts, intromission and ejaculations received multiplied by 100.

2.10 Statistical Analysis

The oestrus period was analyzed by compiling the copulation bouts and excluding the periods in-between. Behavioral duration and frequencies were then divided on the compiled oestrus time for each female to control for the differences in oestrus length. For this thesis I also analyzed the copulation bout where the female was most active, ignoring her other bouts. For a more transparent presentation of change in behavior *during* the episode of the most active copulation bout, I needed to divide that period in shorter units. Since the duration of bouts is not constant for all females, these units cannot be simply based on absolute time. Instead, the time from first lordosis to last lordosis (the length called "copulatory bout" as defined here) was divided by 20 and used that result as the unit of time (as has been done previously by Chu and Agmo, 2014). That means that the first twentieth of a copulation bout would last 24 min in a female with bout duration of 8 h, and 9 min in a female with bout duration of 3 h, just to give two examples. The first twentieth of the bout period is equivalent to the first 5% of that period. In figures and text, I will, for the purpose of clarity, use the cumulative percentage when naming specific intervals of the bout period. Thus, the 5% interval refers to the portion of bout spanning from 0–5%, the 60% interval corresponds to the portion between 55 and 60% of the bout period, and so on. The total number of occurrences or the total duration of behaviors during the 5% intervals was divided by the actual length, in min, of the interval for a particular subject.

A Shapiro–Wilk test showed no homogeneity of variance. All behavioral data were therefore analyzed using the nonparametric Mann–Whitney U test to compare Flx-rats with Ctrl-rats. Friedman's nonparametric ANOVA was used for determining whether there were significant changes in a variable over the 20 5% intervals of behavioral bouts. The Wilcoxon test was used when the different test periods were compared.

Data are always presented as mean ± SEM. All probabilities given in tables, text and figures are two-tailed.

3. Results

3.1 Duration of Behavioral Oestrous

Female rats are normally considered to be in behavioral oestrous whenever they display paracopulatory (darting, hopping, ear wiggling) and receptive (lordosis) behaviors (Uphouse, 2000). Animals not demonstrating such behaviors could therefore be considered not in behavioral oestrus. The females in this present study were manipulated into behavioral oestrus by hormone injections, and as a result all females were in their oestrus period on day seven.

The beginning of oestrus for the Ctrl females (n = 8) ranged from 11:15 h to 18:15 h (M = 12:54 ± 0:48 h) and for the Flx females (n =8) ranged from 10:15 h to 14:15 h (M = 12:47 ± 0:26 h). No significant difference in either start (z = -0.945, p = .382) or length (z = -0.315, p = .798) of oestrus was found for Flx compared to Ctrl females. Figure 4 illustrates the compiled oestrus time for each female.

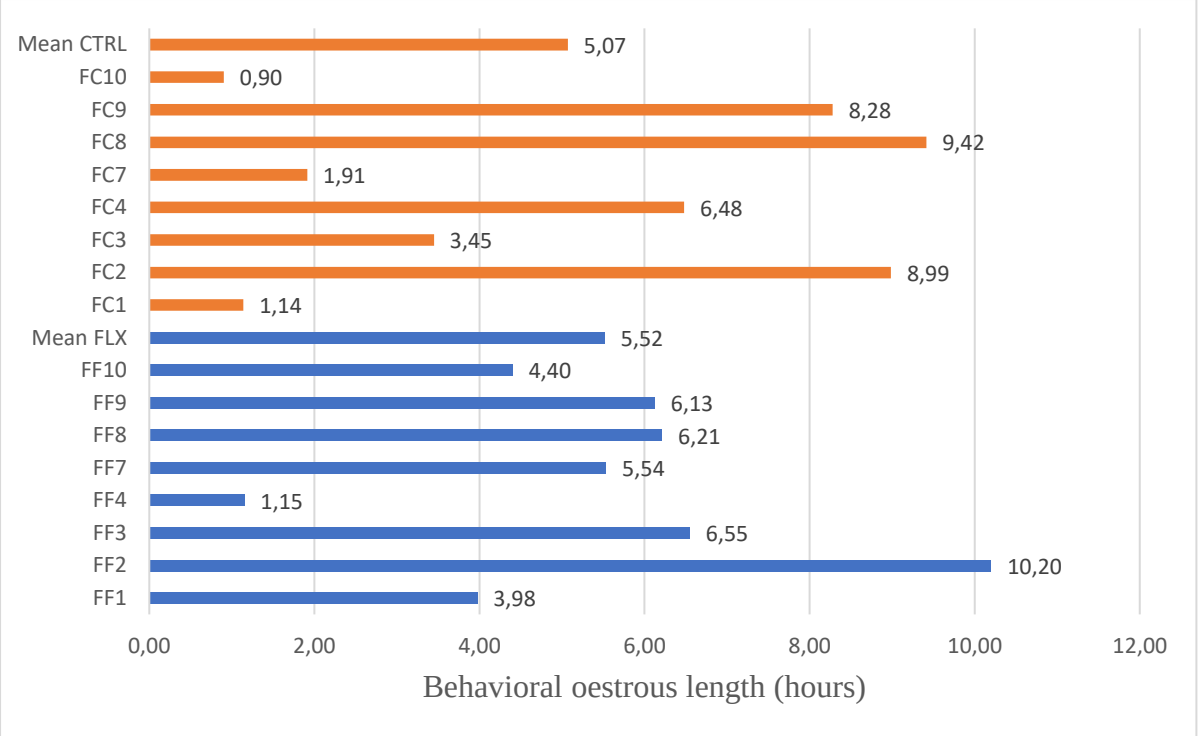


Figure 4. Length of behavioral oestrus in the 16 females in which the entire oestrus periods could be observed (FC = female control, FF = female fluoxetine). Females FC1, FC2, FF1, FF2 belonged to the same cohort, FC3, FC4, FF3, FF4 the same cohort, and so on.

When analyzing the duration and frequency of the behaviors in Table 1, on average none of the social, conflict or sexual related behaviors showed any significant differences between Ctrl and Flx females (*p*s > .37, Appendix B, Table B1). To give an example, Flx females spent the same amount of time on paracopulatory behaviors as Ctrl females (Figure 5). When the total time spent on paracopulatory behavior was divided by the total amount of time spent in behavioral oestrus to control for the differences in oestrous length, no significant differences were found (z = -0.735, p = .505).

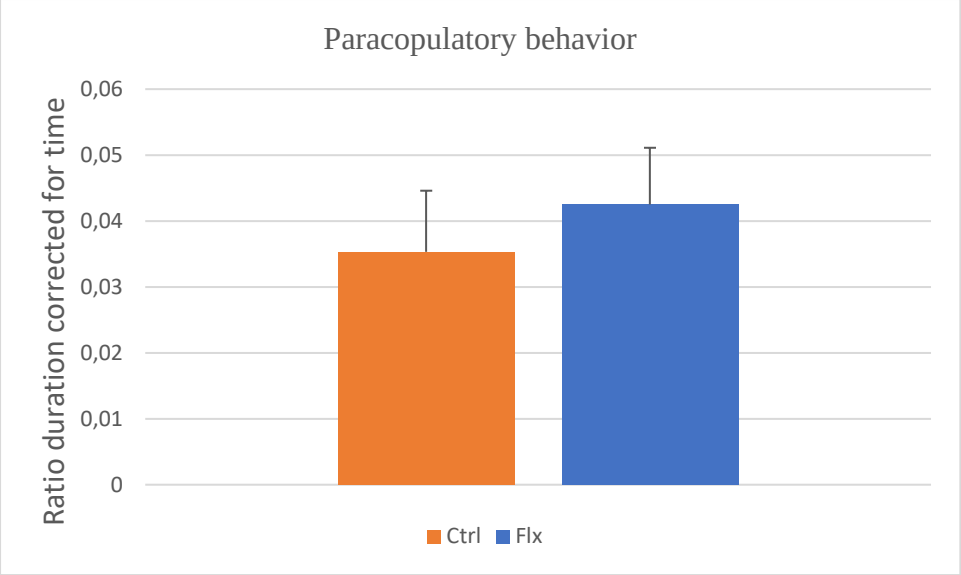


Figure 5. Ratio of paracopulatory behavior where the duration in seconds is divided by length of oestrus duration. Data are mean ± SEM.

As shown in figure 6, there were also no difference in lordosis quotient for Flx females versus Ctrl females in their behavioral oestrus (z = -0.210, p = .878).

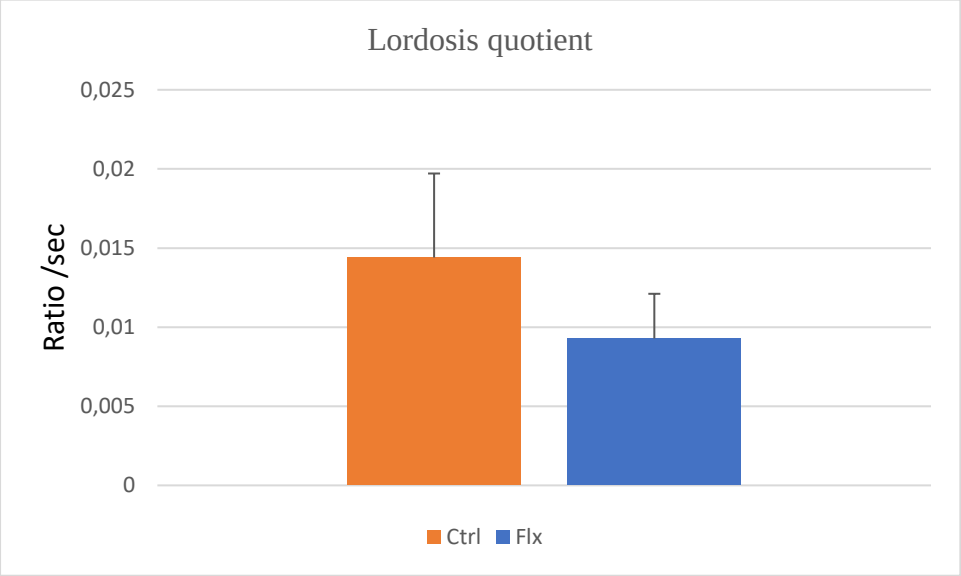


Figure 6. Average LQ ratio for Ctrl versus Flx females when controlling for time spent in behavioral oestrus. Data are mean ± SEM.

When looking at difference in the number of received copulation (mount + intromission + ejaculation) divided by compiled oestrus length, there was no significant

difference for Flx versus Ctrl females (z = -0.210, p = .878) as shown in Figure 7.

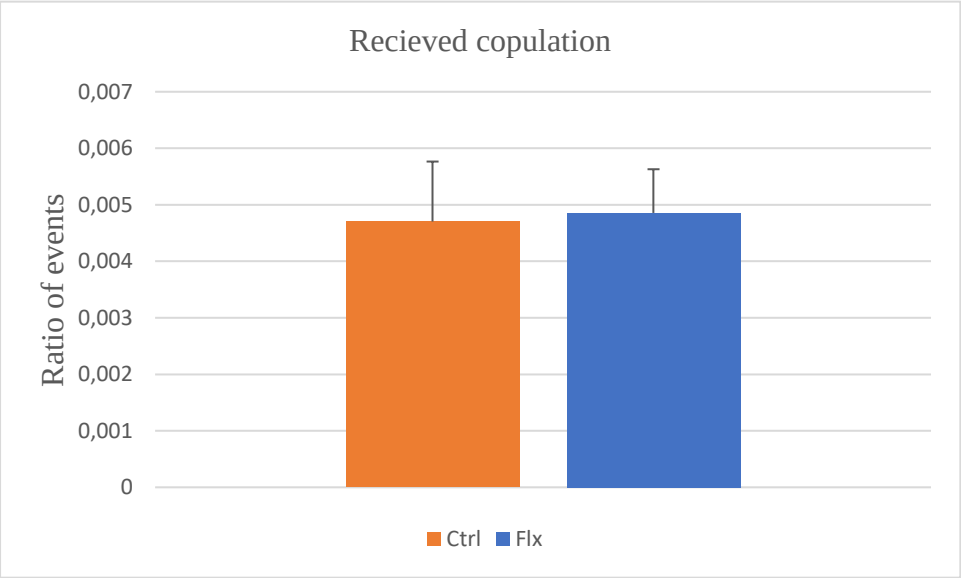


Figure 7. Total number of received mounts, intromissions and ejcaculations for Ctrl versus Flx females when controlling for length of behavioral oestrus. Data are mean ± SEM.

3.2. Duration of Copulation Bouts

I had not originally planned to divide the oestrous period into copulation bouts. In the study by Chu and Agmo (2014), they ended the oestrous period after no visible lordosis for 60 minutes. I intended to use the same defining rule as endpoint, however I soon discovered that the females continued copulation even after multiple 60 minutes windows where no lordosis was shown. After careful consideration with my supervisor, we agreed to treat the episodes that contained lordosis as copulation bouts. Thus, the behavioral oestrus was divided into copulatory bouts, from which the analysis only contains behaviors occurring within those bouts (Eelke Snoeren, personal communication, September 2018).

The number of bouts within a behavioral oestrus was different for each female. There was a minimum of three bouts and maximum of eight bouts for the females, where one bout could be just one lordosis and last a few seconds while the longest bout was 8 hr 15 min (Figure 8). No significant difference in number of sexually active bouts was found between Ctrl vs Flx females (z = -0.109, p = .899). Analysis of behaviors in this thesis stem from the bout containing

the highest frequencies of received sexual behavior, which coincidently is also the longest bout. When presenting this data, durations and number of behaviors are again divided by the respective bouts' duration to control for the differences in time between females. However, the results are the same when the data was analyzed without corrections for time (data not shown).

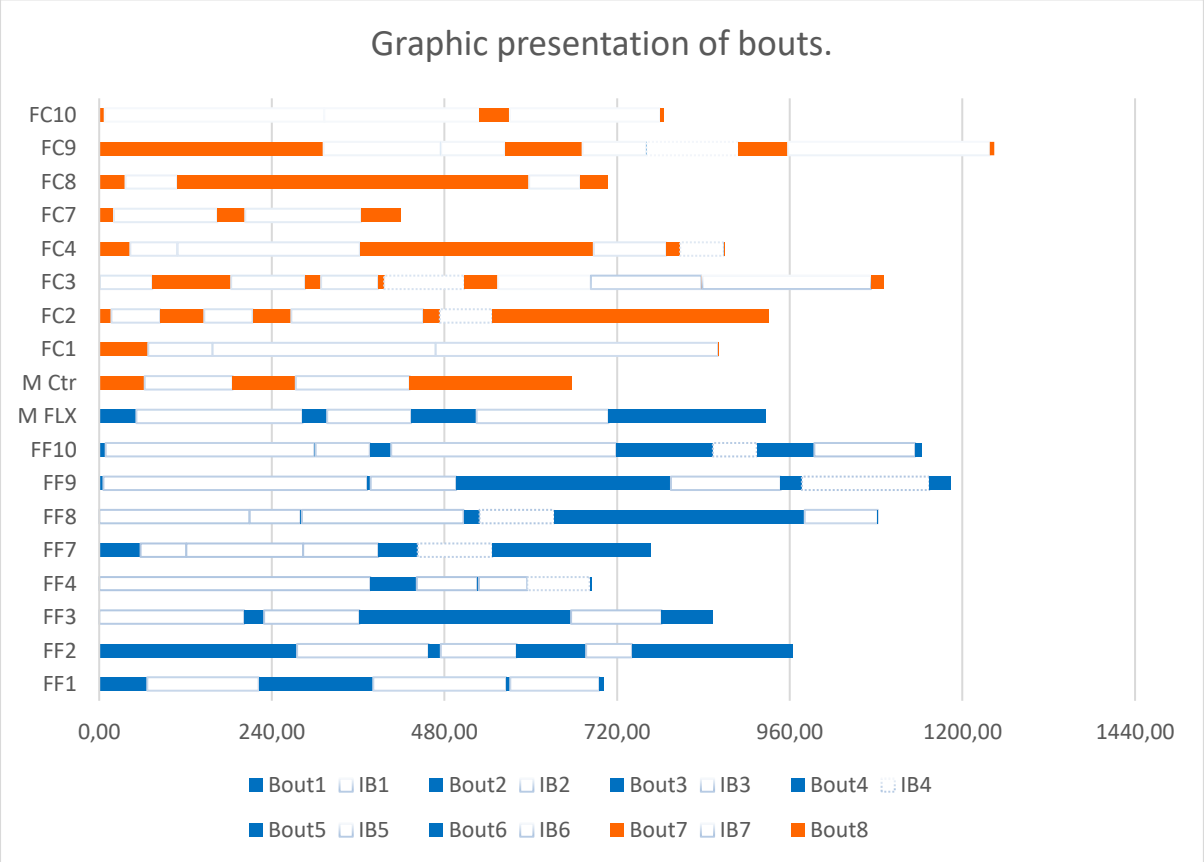


Figure 8. Graphic presentation of each animals' sexual bout. Solid bars represent when one or more lordosis was present *(FC = female control, FF = female fluoxetine, M Ctr = mean for control group, M FLX = mean for Flx group, IB = interbout).* In this graph the start of receptivity is 0,00 for all animals. Time in minutes.

There was no obvious pattern regarding which bouts was the females most active bout, the most active bout could be the first or the fifth bout. No significant differences were found regarding what order their most active bouts were when comparing the groups (z = -0.865, p = .224). Also, when comparing the latency from the P injection to the start of the most active bouts no significant difference between Flx females and Ctrl females was found (M_{Ctrl} = 407 ± 88 min, M_{Flx} = 551 ± 74 min, z = -0.840, p =.442, Figure 9), neither when comparing the lengths

of the most active copulation bout (M_{Ctrl} = 223 ± 38 min, M_{Fkx} = 245 ± 32 min, z = 0.000, p >.99, Figure 10).

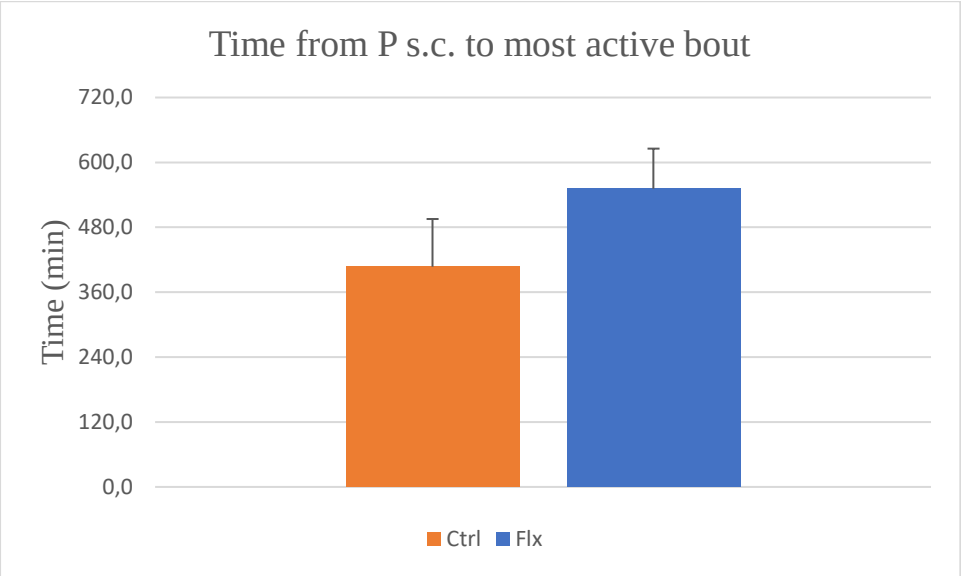


Figure 9. Latency from hormone priming until the most active bout occurred for Ctrl versus Flx females. Data are mean ± SEM.

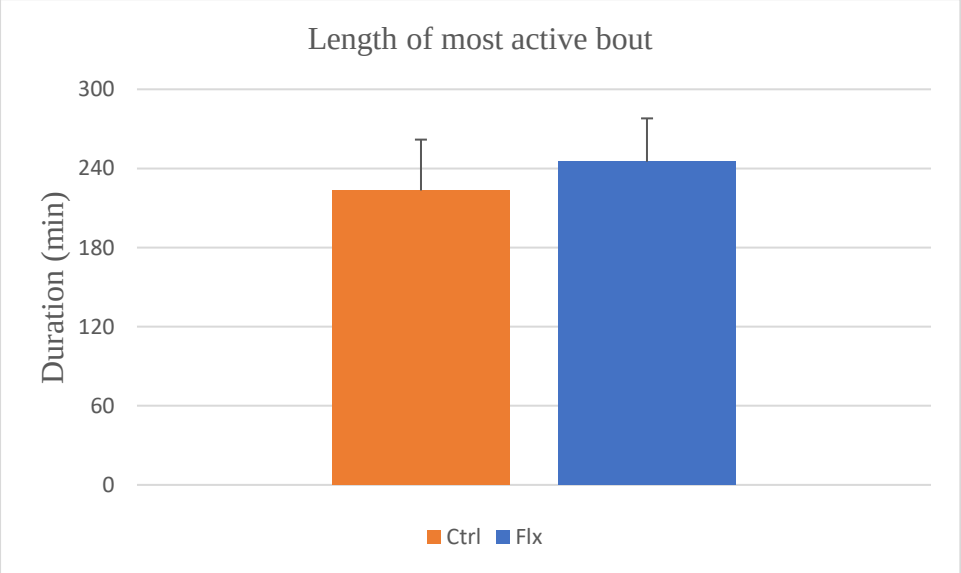


Figure 10. Average length of the bout with most sexual behavior for Ctrl versus Flx females. Data are mean ± SEM.

3.2.1 The most active bout

First, when analyzing the group means for behaviors during the most active bout, there are again no significant differences found between Ctrl and Flx animals. Therefore, I have

displayed the data in appendix C (Table C1). Second, I analyzed the display of the behavior during the most active copulatory bout in more detail by dividing the bout in 5% time-bins and analyzed the behavior within each time-bin (see methods). This was done because in theory there could be periods within these bouts where the groups would be significantly different from each other. For instance, Flx females could have started or ended relatively more active than Ctrl females, resulting in a total time spent (or frequencies) that might not show any differences between the groups. To investigate if this was the case in the current experiment, I therefore ran Friedmans ANOVA on this bout when it was divided in 20 intervals, and I plotted the means for each interval in graphs. One interval was on average 9.26 ± 3.28 minutes for the Ctrl group and 12.26 ± 1.64 minutes for the Flx group. I will present the data and analysis comparing between groups for each interval with a Mann-Whitney U test and then within groups using Freidmans ANOVA for social behaviors first, then conflict behaviors and last the sexual behaviors during the most active bout. An overview of the results from the statistical analysis is found in appendix D, Table D1.

As shown in Figure 11, Ctrl females seem to have a slight increase in time spent sniffing other rats during the 15% - 60% interval in their most active bout, while the Flx females show less variation in time spent sniffing other rats for every interval. After analyzing if there are any intervals where the groups could be different compared to each other, no intervals were found significant (the interval with the lowest p-value is the 5% interval, $z = -1.904$, $p = .058$). When looking at the change in behavior over time within one group, there is no significant difference for Ctrl females ($\chi^2 = 21.36$, $df = 19$, $p = .317$) or Flx females ($\chi^2 = 18.26$, $df = 19$, $p = .505$) regarding sniffing others (Figure 11).

Sniffing of the anogenital region show a spike in time spent at the 25% interval for the Ctrl animals, while the Flx animals have three spikes in time spent which occurs at the 35%, 80% and 95% interval. There is no significant difference between the two groups, however the

5% interval (z = -1.991, p = .063) and the 70% interval (z = -2.208, p = .077) show a trend. Changes in behavior over time within each group was not significant for Ctrl females (χ^2 = 25.45, df = 19, p = .146) or Flx females (χ^2 = 28.23, df = 19, p = .079) data shown in Figure 12.

After combining sniffing others, anogenital sniffing of others and grooming others to one parameter; social behavior, we see that the spikes in time spent on social behavior flattens out for both groups. When inspecting figure 13 one might get the impression that the Ctrl females spend more time on social behavior from the 15% interval and until the 65% interval compared to Flx females. Analysis of each interval showed only one significant difference between the two groups, the 10% interval where Ctrl females spent shorter time on social behavior compared to Flx females (z = -2.102, p = .035). When comparing change in social behavior over time within each group Friedmans ANOVA did not show a significant difference for Ctrl females (χ^2 = 22.99, df = 19, p = .238) or Flx females (χ^2 = 19.23, df = 19, p = .442).

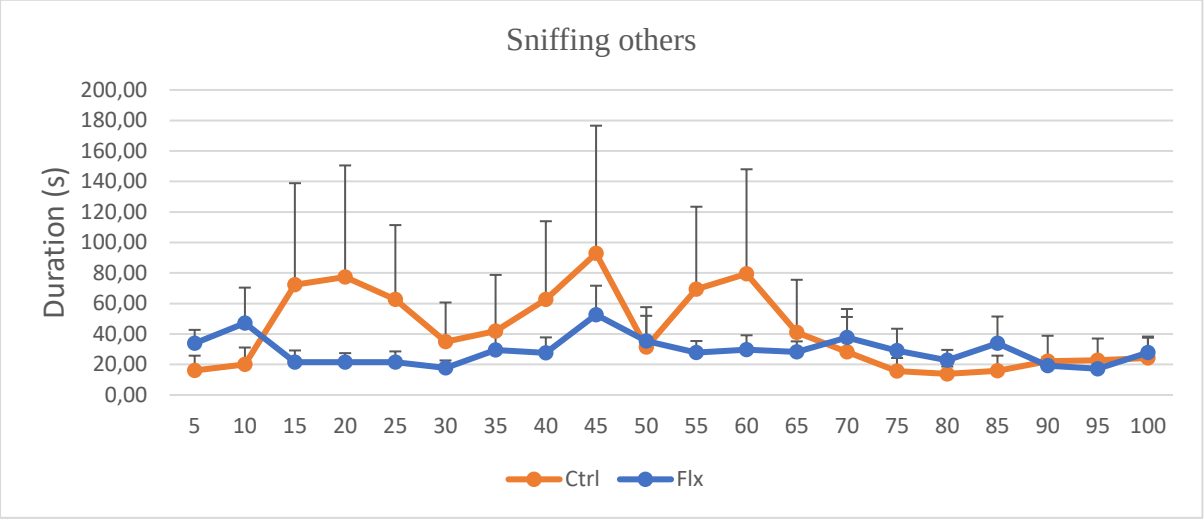


Figure 11. Time spent sniffing others (s/min) during the most active bout. Ctrl = control females, Flx = fluoxetine exposed females. Data are mean ± SEM.

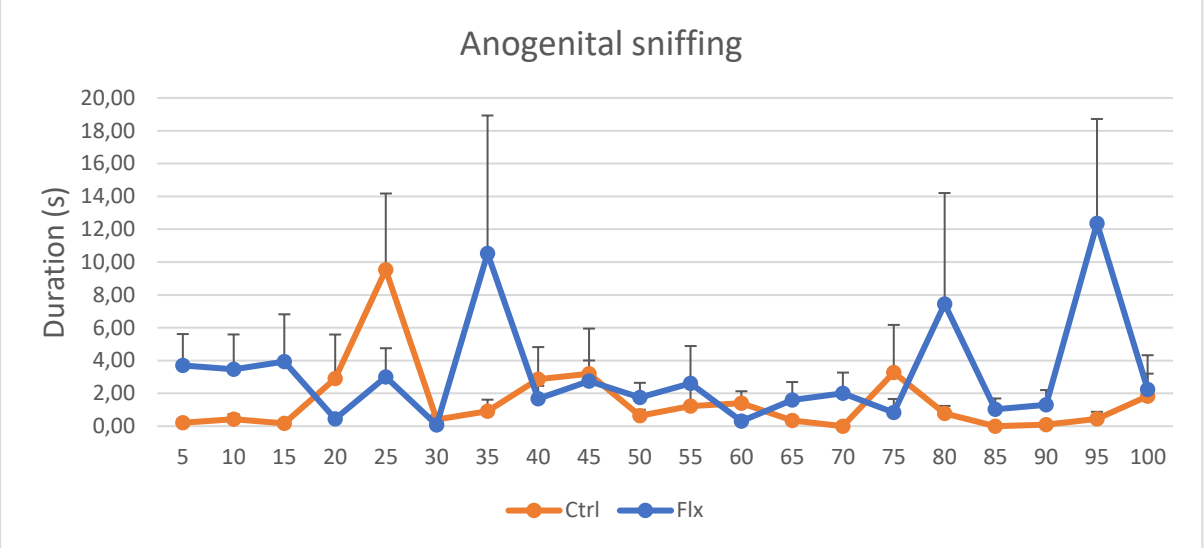


Figure 12. Time spent sniffing the anogenital region of others (in s/min) during the most active bout. Ctrl = control females, Flx = fluoxetine exposed females. Data are mean ± SEM.

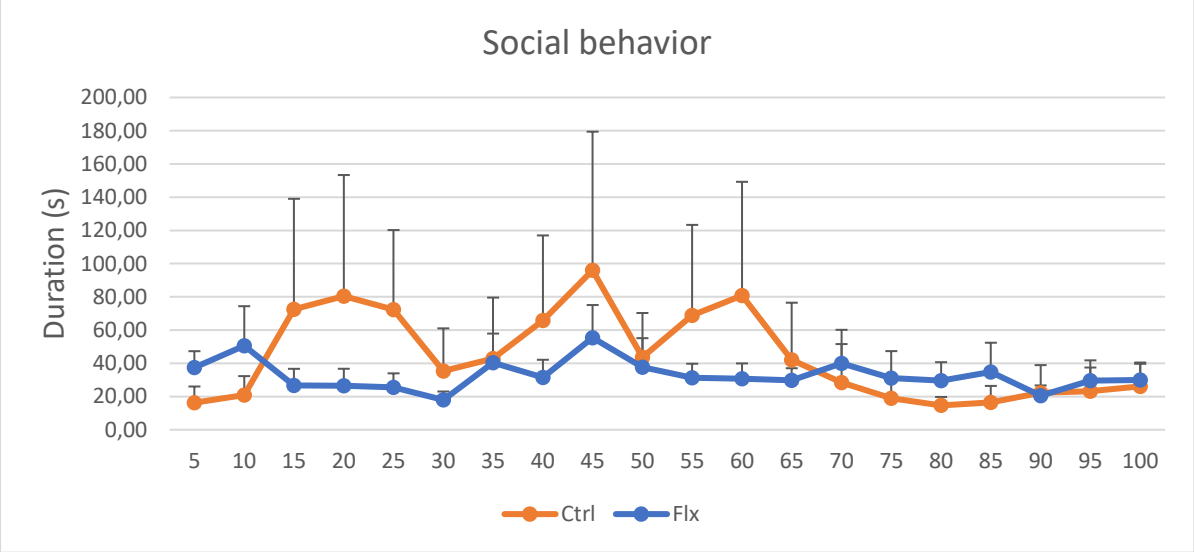


Figure 13. Time spent on social behavior (in s/min) during the most active bout. Ctrl = control females, Flx = fluoxetine exposed females. Data are mean ± SEM.

Ctrl females seem to spend longer time in conflict behaviors (boxing/wrestling, nose-off and rejection) than the Flx females in the start if the active copulatory bout (first time bins) up until the 30% interval (Figure 14). The amount of time spent in conflict behavior for Ctrl females fluctuates from the start until the 70% interval, after which they show more stable level of conflict behavior until the end. A closer inspection of the graph (Figure 14) shows an increase in time spent in conflict behavior starting at the 35% interval for the Flx females, then

a dip at 55% and a slow decent toward the 90% interval. However, after analyzing if there was a difference between the two groups, no intervals were significantly different, although the 55% interval indicate a trend (z = -1.706, p = .099). Interestingly, upon analysis the total change in conflict behavior within the Flx female group was found to be significant (χ^2 = 30.69, df = 19, p = .044) while there was not a significant change in the conflict behavior for the Ctrl females (χ^2 = 13.12, df = 19, p = .832).

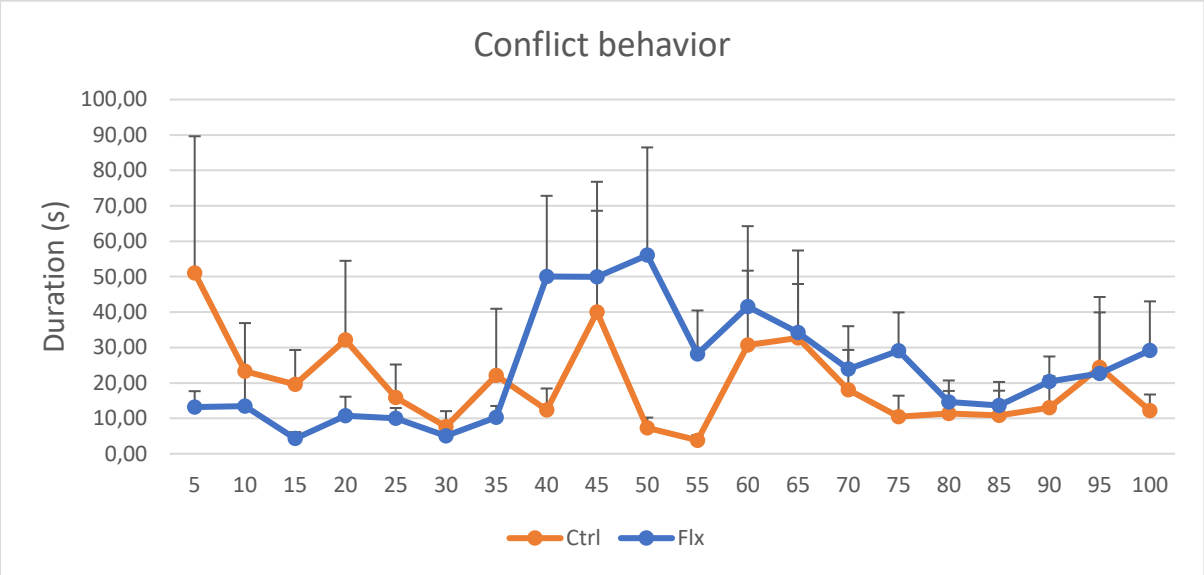


Figure 14. Time spent in conflict with other rats (in s/min) during the most active bout. Ctrl = control females, Flx = fluoxetine exposed females. Data are mean ± SEM.

Both groups spend the same amount of time doing paracopulatory behavior of rather high durations that slowly decline from the start of the most active bout until the 20% interval (Figure 15). Then, the Ctrl females spend less time doing paracopulatory behaviors compared to Flx females until the end. In comparison, time spent doing paracopulatory behaviors for the Flx females show an increase at the 25% interval, which then slowly declines towards the 65% interval. After analyzing these intervals to detect differences between the groups, results show that the 75% interval was significantly different between the groups; in this interval the Flx females spent longer time doing paracopulatory behaviors than Ctrl females (z = -2.426, p = .015). Within the Ctrl group, the time spent doing paracopulatory behavior show an irregular

pattern going up and down for the duration of the bout, and this change was significant (χ^2 = 34.1, *df* = 19, *p* = .015). When looking within the Flx group, time spent doing paracopulatory behavior first has a dip from the start to the 20% interval, then an increase at the 25% - 30% intervals, and a slow decline from there until the 70% interval. Again, the Friedmans ANOVA analysis for the Flx females resulted in a non-significant change in behavior within this group (χ^2 = 15.81, *df* = 19, *p* = .670).

Not entirely surprising, almost identical patterns are repeated for the received copulation behaviors (mount + intromission + ejaculation, shown in Figure 16) when looking at corresponding paracopulatory behavior for the respective groups (paracopulatory is shown in Figure 15). In the 40% interval the Flx females receive significantly more copulative behaviors towards them versus the Ctrl (*z* = -2.493, *p* = .018), the same is repeated in the very last interval where the Flx group receive more copulative behaviors than the Ctrl group (*z* = -2.031, *p* = .047). The change in received copulation is not significant within the Ctrl group (χ^2 = 21.76, *df* = 19, *p* = .296), and the plotted datapoints for behavior that could resemble the shape of an M is trending for change within the Flx group (χ^2 = 29.27, *df* = 19, *p* = .062), data shown in Figure 16.

When we look at the number of lordoses the Ctrl group display for each interval, it is hard to observe any pattern (Figure 17). Even if this is the groups most active bout, the number of displayed lordoses fluctuates from one interval to the next and might be lower compared to Flx females. On the other hand, the Flx females after first showing a decline, they have an increase in lordosis response from the 25% interval and to the 85% interval. In these intervals the Flx group show higher response numbers than the Ctrl group. Significant differences between the groups are found in the 30% interval (*z* = -2.052, *p* = .042), the 40% interval (*z* = -2.414, *p* = .018), the 50% interval (*z* = -2.194, *p* = .029) and the 100% interval (*z* = -2.414, *p* = .019). For the change in lordosis behavior (Figure 17) during the most active bout, both

groups when compared within subjects have trending results, Ctrl females (χ^2 = 28.78, *df* = 19, *p* = .070) and Flx females (χ^2 = 29.48, *df* = 19, *p* = .059).

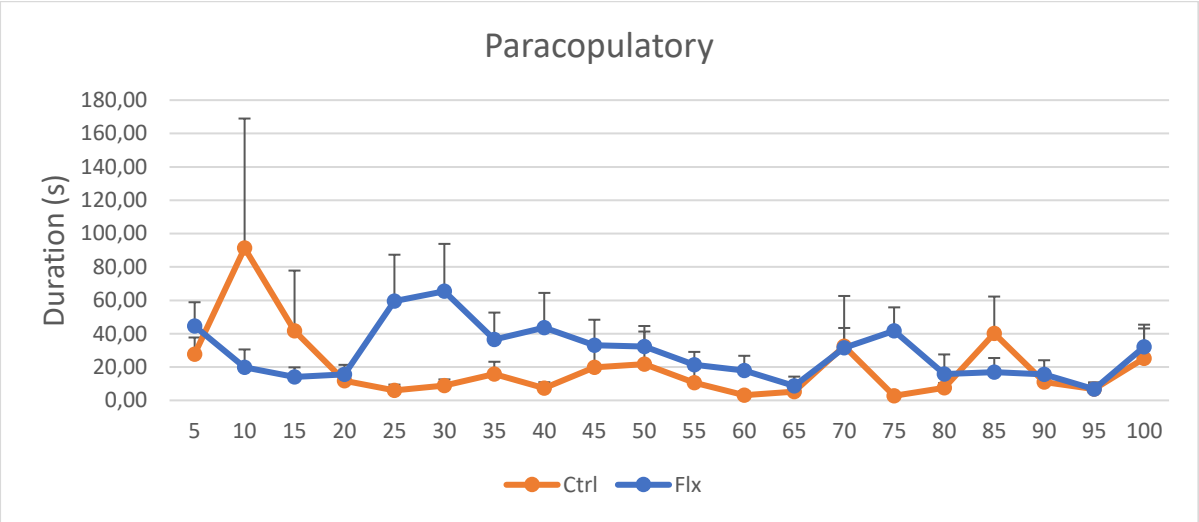


Figure 15. Time spent doing paracopulatory behavior (in s/min) per 5% interval during the most active bout. Ctrl = control females, Flx = fluoxetine exposed females. Data are mean ± SEM.

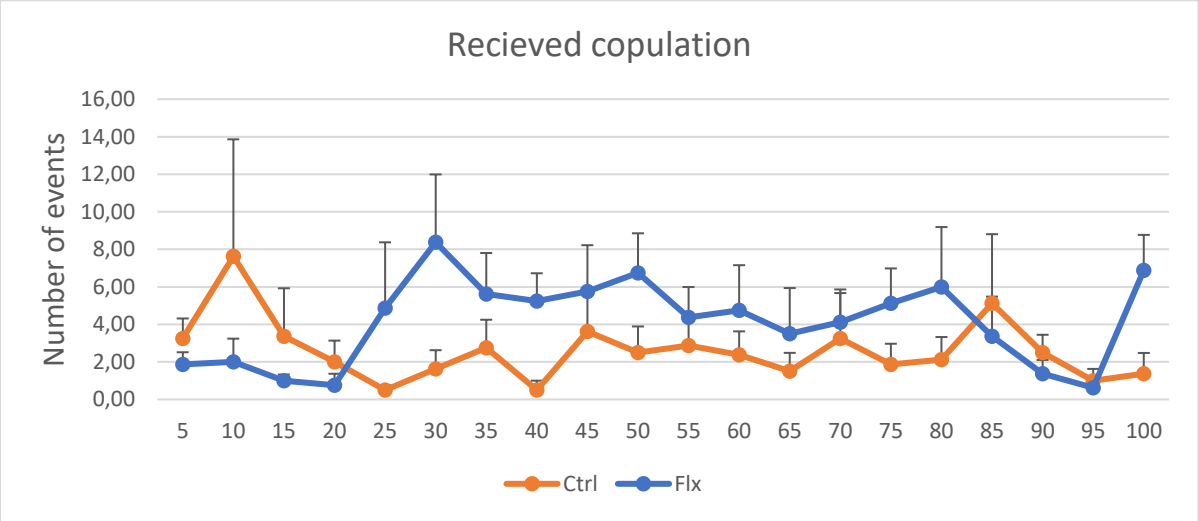


Figure 16. Frequency of received copulation for the most active bout. Ctrl = control females, Flx = fluoxetine exposed females. Data are mean ± SEM.

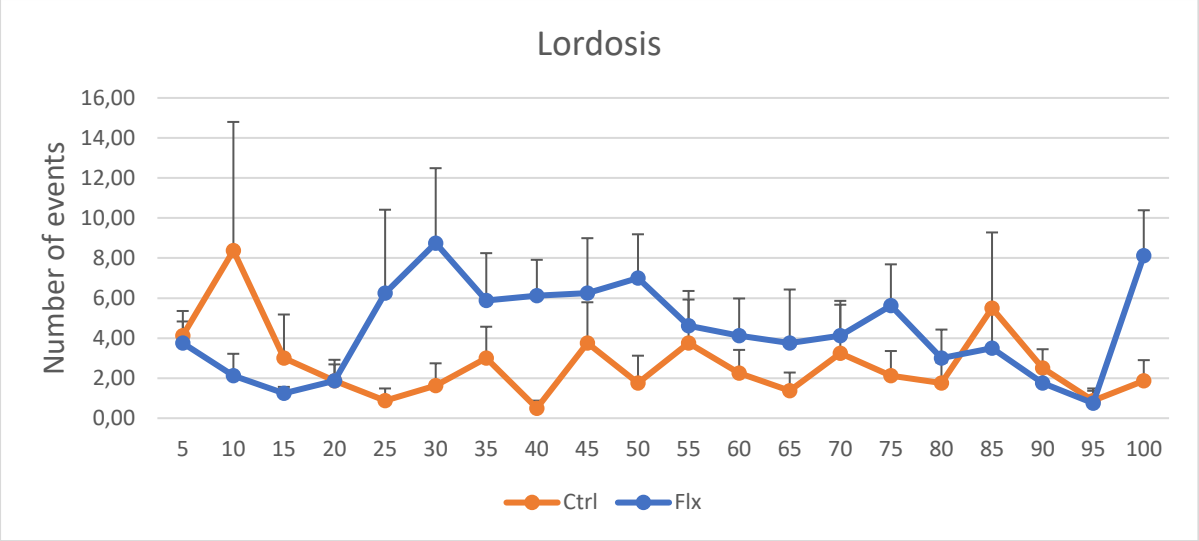


Figure 17. Frequency of displayed lordeses for the most active bout. Ctrl = control females, Flx = fluoxetine exposed females. Data are mean ± SEM.

4.Discussion

The current study showed that Flx females displayed the same patterns of sexual behavior as Ctrl females, suggesting that perinatal Flx exposure does not affect female sexual functioning. This finding contradicts my expectations. -The first expectation was that the Flx exposed females would show higher levels of paracopulatory behavior compared to the Ctrl group. Based on my results showing no significant differences, this expectation is not met for the total oestruos period, nor for the most active bout. A detailed look at the most active bout when divided into intervals revealed only one interval where the Flx females showed significantly higher durations of paracopulatory behavior compared to the Ctrl group (70% interval). However, although the graph visually seems to indicate that the Flx females might spend longer time on paracopulatory behaviors than the Ctrl females, this effect is not significantly different.

My second expectation, a higher number of lordoses for Flx females compared to the Ctrl females was also not backed by the data. On average, the number of lordoses (also when controlled for the oestrus length) is not different between the groups in both the total oestrus period and during the most active bouts. There are some intervals where the Flx females have

significantly more lordoses than the Ctrl group, however this might be due to the Ctrl females having lower than usual numbers in those intervals rather than Flx females having more. Given that the most active bout for each female occurs at different times during the 7th day, it is difficult to state what causes these dips for the Ctrl group. For instance, one easily refutable explanation might be that the males are under fatigue. There are four males per cohort and furthermore there are four different cohorts, thus the odds for all males in all cohorts *not* copulating on only the Ctrl females in those intervals are too small. At the same time, it is also difficult to attribute a cause to the females themselves, since all females were primed with hormones into an optimal state for sexual receptivity (Spiteri et al., 2010). Therefore, they should theoretically not show such sub-optimal performances. In comparison, what causes the sudden increase in lordoses response for the Flx group, shown in the last interval (Figure 17), is also puzzling. When comparing the graphic representation for the paracopulatory behavior in figure 13 to the numbers of lordoses in figure 15, there is a nice relationship between these two behaviors. Usually copulation follows paracopulatory behavior, resulting in a lordosis. Therefore, paracopulatory behavior could indicate what level of lordoses responses each group would display, akin to having predictive power. Thus, when we see an increase in paracopulatory behavior for one interval we can expect a higher number of lordoses in the same or the following interval. However, in the last intervals for paracopulatory behavior (Figure 15) the amount of paracopulatory behaviors does not match the number of lordoses shown.

The expectations regarding the differences in oestrus periods was obviously not met. This is based on the data showing no overall difference between the groups in their time spent and numbers of any of the behaviors that I scored. This might arguably not be a robust conclusion based on the discovery that the animals in this study copulated in bouts. However, I did expect an earlier start and/or a start with higher intensity for the Flx group compared to the Ctrl group. Thus, if we look at the presence of the bouts there are no significant differences

in number of bouts or total length for all bouts for the two groups, indicating that one group does not copulate longer that the other. In addition, based on my expectation for the total oestrus period there are no differences when looking at which bout was the most active bout, the Flx group would be expected to have their most active bout earlier than the Ctrl animals. To follow this up, there is on average no significant difference in latency to the different groups' most active bout and no significant difference in this bout's length.

Our data was in line with a previous study showing that perinatal fluoxetine exposure does not affect sexual behavior in rats (J. D. Olivier, Valles, et al., 2011), however this study reported the effects on male instead of female sexual behavior. In addition, that study chose to inject a different dose of fluoxetine compared to our study (12 mg/kg/day versus our 20 mg/kg/day) and their exposure period was from gestational day 11 until the pups were born compared to our perinatal exposure period (gestational day 0 until postnatal day 22). When combining the results reported by J. D. Olivier, Valles, et al., (2011) with this current study we see a strengthened argument for no effect of developmental fluoxetine exposure on the basis that there was no effect observed in both sexes.

Our data was in contradiction to some other studies that reported stimulating effects of early life exposure to SSRIs on sexual behavior (Maciag et al., 2006; Rayen et al., 2014). Upon closer inspection there are some methodological differences between the existing literature and our study. As a consequence, these differences and their significance will be discussed. These studies either used a different rat strain (Sprague-Dawley or Long-Evans), used intact females, different method (minipump or s.c injections), SSRI (fluoxetine or Citalopram) and dosage of SSRI administered (5mg/kg/day) and a standardized copulation test (either 45 minutes or 60 minutes). Maciag et al. (2006) found a decrease in male sexual behavior whereas in contrast to this Rayen et al. (2014) found an increase in female sexual behavior. First of all, the choice of route for administering the SSRIs could have caused the

observed differences in effect, which could be due to the relevance of stress caused by injecting the pups. In the literature we find reports that stressing pregnant mothers before birth or stressing the pups after birth can have severe effects on behavior later in life (Nelson, 2011, p. 598 – 601). By having such knowledge, we see that when opting for subcutaneous injections to either mothers or pups, although the method grants accurate doses it also increases stress thus, s.c injections might introduce confounders in prenatal and perinatal designs. On the other hand, it may be more convenient to have a mini pump implanted under the skin of pregnant rats compared to giving them oral gavage every day, again the daily treatment could stress the dams. However, whether the rats receive one spike of FLX once a day in their stomach or have a more even dose of the drug distributed during the day could impact the results. Although the minipumps might reduce stress, the oral administration route to a greater extent mimics how humans take SSRIs. While it might not be as accurate as the mini pump, it still ensures that the fetuses are exposed to the drug (J. D. Olivier, Valles, et al., 2011; Rayen et al., 2014).

Besides the route of administration, the different rat strains that scientists use could also have caused the different results. This would hardly be a problem if all the results pointed in the same direction, however when there is observed effects in one strain, for instance increased female copulation in Sprague-Dawley, and no observed effect on female copulation in Wistar, then our ability to conclude on this matter is weakened. Not too often do we see two strains of rats used in one study, although Jones, Ismail, and Pfaus (2017) did use Long-Evans together with Wistar in their study. Therefore one of their discussion points were speculations on differences between OVX Long-Evans and OVX Wistar regarding their sensitivity to EB priming (Jones et al., 2017). In relevance to this experiment it might indicate that the high dose of EB, P or both together, when the Wistar strain is used, thus cancels out the expected effect of perinatal fluoxetine exposure. This topic is hard to elaborate further

upon since most of the previous research focuses on males and male behavioral and less on the female side (Glover & Clinton, 2016; Mendelson, 1992; Uphouse & Guptarak, 2010). To sum this up, we are still in the need of more research on female sexual behavior in rats, because the previous literature on this topic does raise more questions than it provides answers.

On the notion that we need more research on female behavior, a different problem might unearth itself. Could there be different observable effects when using OVX rats versus intact rats that are primed with E? In this study, the discovery that OVX primed rats observed in the seminatural environment copulated in bouts was quite the surprise. Especially given that Chu and Agmo (2014) described the existence of a behavioral estrus consisting of only one copulatory bout, therefore we expected to see the same pattern in OVX females. In contrast, our data indicated that OVX females primed with hormones seem to copulate in copulatory bouts forming one behavioral oestrus period. Initially, I presumed that what I was seeing was "accidents" of copulation. For instance, where one female was doing paracopulatory behaviors towards a male, she would jump over another female, and thus this unlucky female would be mounted by the male in pursuit. Some of the bouts in the data from this study are examples of such behaviors, whereas this is not the case for all of the bouts I scored. If we look at Figure 8, clearly some of the bouts are much longer than a simple mount and go from the male. Thus, we see the other example, -when some females do re-initiate a copulation bout, where some of these bouts are of same length as traditional copulation tests. In theory I could have analyzed the data from these shorter bouts and compared that data to other articles who only used standardized copulation tests and then see how the data compared. However, such thoughts should be discouraged on the basis of what behaviors those shorter bouts might contain, one could speculate that that maybe the shorter bouts include more errors in them. To give an example, if the bout is created by a somewhat irregular behavior where a male is raping the

female. In that case there would probably not be a lot of paracopulatory behavior present. Thus, when analyzing such a bout the female would bring in almost “normal” levels of mounts and lordosis while also showing lower levels of paracopulatory behaviors. By selectively sampling only the “weird” bouts for analysis I would bias myself and as a result make it easier to conclude on an effect that is not present, especially after knowing the bigger picture. On such a notion a more accurate result is obtained by looking at the longer bouts of the females, due to sampling behaviors over a longer time period. In addition, this thesis only looked at the most active bout, ensuring that the females in fact were willing to copulate. In contrast to this is standardized copulation tests, where such individual differences will be neglected.

The same argument can be made against our decision on how to compare the groups. How correct the method of combining different animals' most active bouts on top of each other, to create the most active bout for each group, and then further claiming that we can treat these bouts as equal, might not be optimal. When looking at Figure 8, we see that the bouts are nicely spread out over the length of behavioral oestruos. The animals are thus compared on relative terms and not at a fixed time interval. From the viewpoint that every animal is hormonally primed at the same time and therefore theoretically they should show less variation in when their most active bout occurs, the observed data might dispute this. Interestingly the groups do not show significant differences in when they start their most active bout.

Returning to the question regarding possible differences in OVX versus intact females, what observed differences can we find? One of the most studied behaviors is the lordosis response and in natural cycling females the display of lordoses usually signals that they are receptive. By comparison, OVX females do not display any sexual related behaviors unless they are primed with hormones. Could there be side effects of priming that are not yet observed? If we look into the article by Chu and Agmo (2014), they report that the longest interval between one lordosis and the next was 17 minutes in intact females. A different pattern

emerges if we look at Figure 8 in the present study. Here it is noticeable that unsolid bars indicate at least a period of + 1 hour where no lordosis was visible. However, this does not indicate that these rats are incapable of displaying lordoses within that period. Chu and Agmo (2014) had natural cycling rats, without any estradiol priming to induce oestrus, while this study OVX'ed the females two weeks prior to entering the seminatural environment. Therefore, it is tempting to speculate that one of the underlying causes behind the different copulation patterns could be the OVX + hormonal priming. It has proven difficult to find literature where the authors have investigated (i) how long OVX rats which later are primed with EB + P are receptive and (ii) what copulation pattern they might have.

Most of the earlier studies on female receptivity used intact animals while fewer studied OVX animals. Such discrepancies could stem from the classic endocrine presumption that when the missing hormones are re-administered, there should not be too many differences compared to intact animals. What reports regarding sexual behavior was found that could explain the copulation pattern in this study? Kuehn and Beach (1963) used a paradigm where they tested Long-Evans rats hourly to describe changes in female behavior and receptive length. Intact female animals were submitted for a copulation test every hour with a sexually experienced male. The receptive period started when the female had displayed two lordoses in two successive tests and it was ended when the female failed to show a lordosis in response to a male mount in two successive tests. Due to the temporal pattern enforced by the paradigm the authors acknowledge that their reported average receptive length for the females of 19.7 hours might not be correct. In addition, it is plausible that the short duration of the tests, 90 seconds after the first mount (Kuehn & Beach, 1963, p.288), might not control for where the male is in his cycle, and thus creating "bouts" which consists of more mounts and/or intromissions than needed for an ejaculation. Male rats need about 10 to 20 intromissions during a short interval (2 – 10 minutes) in order to ejaculate, and it is possible that the constant

change in females disrupts his copulation pattern (Snoeren et al., 2014a). For instance, in the present study there are multiple males present, and therefore it is possible that one male may have started his copulation first on one female and then he switches to a different female. Such a case would probably not show up in the graph of figure 6, if the female he started on was already initiated on by a different male. What instead would show was a shorter bout for the female this male switched too, since this male already had started his copulation bout, or it would create two short bouts for both females.

The data provided by Kuehn and Beach (1963) do indicate that it is possible to influence the receptive length in natural cycling animals, especially if compared to the length of the females in Chu and Agmo (2014). Two other studies (Ball, 1937; Blandau, Boling, & Young, 1941) on receptivity reported longer receptive durations than Chu and Agmo (2014). Both studies have the hourly test interval like Kuehn and Beach (1963) and use intact female rats. Ball (1937) argues that the smear taking to determine vaginal cycles prolongs the length of the rats' receptive cycle. As previously mentioned, when the researchers are handling the animals this will often increase stress levels in the rats, and there should be no reason not to believe that the method of extracting the blood from the vagina of the females also induces stress. Therefore one should consider caution when comparing the results of Ball (1937), Blandau et al. (1941) and Kuehn and Beach (1963) to the results from Chu and Agmo (2014), since the use of the seminatural environment beautifully deals away with such concerns and therefore may show a more transparent picture of receptive length in females.

Whatever the reason for the copulation pattern observed by the researcher it seems not to coincide one to one with the hormone levels in the rat. In the case of the intact female rat the length of the receptive period is malleable as shown by (Kuehn & Beach, 1963), and furthermore the termination of the oestrus period does not show a decline in behaviors as the hormonal levels drop (as argued Chu and Agmo, 2014). The present study further complicates

this picture. Priming OVX females with one single dose of E and then later with P should not have created such fluctuating patterns consisting of bouts. We do know that these hormones need to be present in the animal for it to show both paracopulatory and lordosis behavior, however how the observed periods of zero copulation reported in this study ties to the endocrine state of the animal is puzzling. Thus, further research is required to provide better answers to the question.

Nevertheless, an explanation for why the rats in the present study copulate in bouts might still exist. One conspicuous comment not yet touched upon involves the willingness of the females. To start off on one point, an indirect measure of willingness is the amount (or lack) of conflict displayed from the females towards the males. There is arguably little conflict observed in the periods where there is no copulation, most of this time is spent on resting (data not shown). In contrast, when comparing the increase in copulation for Flx females (shown in Figure 14) to when the increase in conflict is (Figure 12), the intervals do not coincide. Thus, when the male's interaction with the females increases, the females do not respond towards the male's increased interests with conflict behaviors. On such a notion the females might instead be willing to copulate, and a proposed conflict explanation can be refuted.

A more direct measure of willingness is found in the paracopulatory behavior. Do the rats in this study neglect to show such behaviors in between the bouts? Due to the creation of copulation bouts, the data in between these bouts is excluded from the data analysis in this study. However, all the rats show paracopulatory behavior during inter-bout periods, in addition some rats even display this after their final lordosis is seen. Arguably only the seminatural environment discloses such observations and that is exactly what this study did. By default, a seminatural environment enforces zero constraints on the behaviors of the animals within it, and the researcher can observe whatever behavior the animal choses to display. In

the light of such an argument care should be taken when proposing a rudimentary motivational explanation for the copulation pattern observed.

One thing is certain, the bouts seen in this study cannot be caused by the SSRI exposure on the simple fact that the Ctrl group also show this copulation pattern. Thus, we can fully conclude that perinatal SSRI exposure does not influence the sexual behavior at adulthood.

Conclusion

Based on the data in the current experiment there is no effect of perinatal fluoxetine exposure to fetuses and later effects on their sexual behavior as fully primed OVX female Wistar rats in adulthood. Where previous literature has relied on simple standardized copulation tests to document their effects on female sexual behavior, new studies emerge demonstrating how narrow insights those studies yield. Thus, future research should make haste in implementing proper methods when investigating sexual behavior.

References

Agmo, A. (2011). *Functional and dysfunctional sexual behavior: a synthesis of neuroscience and comparative psychology*: Academic Press.

Albert, D. J., Jonik, R. H., & Walsh, M. L. (1992). Hormone-dependent aggression in male and female rats: experiential, hormonal, and neural foundations. *Neurosci Biobehav Rev, 16*(2), 177-192.

Ball, J. (1937). *A test for measuring sexual excitability in the female rat*: Johns Hopkins Press.

Blandau, R. J., Boling, J. L., & Young, W. C. (1941). The length of heat in the albino rat as determined by the copulatory response. *Anatomical Record, 79*(4), 453-463. doi:10.1002/ar.1090790405

Chu, X., & Agmo, A. (2014). Sociosexual behaviours in cycling, intact female rats (Rattus norvegicus) housed in a seminatural environment. *Behaviour, 151*(8), 1143-1184. doi:10.1163/1568539x-00003177

Frye, C. A., & Walf, A. A. (2004). Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. *Behavioral neuroscience, 118*(2), 306.

Glover, M. E., & Clinton, S. M. (2016). Of rodents and humans: A comparative review of the neurobehavioral effects of early life SSRI exposure in preclinical and clinical research. *Int J Dev Neurosci, 51*, 50-72. doi:10.1016/j.ijdevneu.2016.04.008

Heijkoop, R., Huijgens, P. T., & Snoeren, E. M. S. (2017). Assessment of sexual behavior in rats: The potentials and pitfalls. *Behav Brain Res*. doi:10.1016/j.bbr.2017.10.029

Hliňáck, Z. (1993). Social recognition in ovariectomized and estradiol-treated female rats. *Hormones and behavior, 27*(2), 159-166.

Houwing, D. J., Heijkoop, R., Olivier, J. D. A., & Snoeren, E. M. S. (2019). Perinatal fluoxetine exposure changes social and stress-coping behavior in adult rats housed in

a seminatural environment. *Neuropharmacology, 151*, 84-97. doi:10.1016/j.neuropharm.2019.03.037

Jones, S. L., Ismail, N., & Pfaus, J. G. (2017). Facilitation of sexual behavior in ovariectomized rats by estradiol and testosterone: A preclinical model of androgen effects on female sexual desire. *Psychoneuroendocrinology, 79*, 122-133. doi:10.1016/j.psyneuen.2017.02.018

Kuehn, R. E., & Beach, F. A. (1963). Quantitative Measurement of Sexual Receptivity in Female Rats 1. *Behaviour*, *21*(3-4), 282-299.

Maciag, D., Simpson, K. L., Coppinger, D., Lu, Y., Wang, Y., Lin, R. C., & Paul, I. A. (2006). Neonatal antidepressant exposure has lasting effects on behavior and serotonin circuitry. *Neuropsychopharmacology*, *31*(1), 47. doi 10.1038/sj.npp.1300823

McClintock, M. K., & Adler, N. T. (1978). The role of the female during copulation in wild and domestic Norway rats (Rattus norvegicus). *Behaviour*, *67*(1), 67-95.

McClintock, M. K., Anisko, J. J., & Adler, N. T. (1982). Group mating among Norway rats II. The social dynamics of copulation: competition, cooperation, and mate choice. *Animal Behaviour*, *30*(2), 410-425.

Mendelson, S. D. (1992). A review and reevaluation of the role of serotonin in the modulation of lordosis behavior in the female rat. *Neurosci Biobehav Rev*, *16*(3), 309-350. doi:https://doi.org/10.1016/S0149-7634(05)80204-0

Olivier, B., Chan, J. S., Snoeren, E. M., Olivier, J. D., Veening, J. G., Vinkers, C. H., . . . Oosting, R. S. (2010). Differences in sexual behaviour in male and female rodents: role of serotonin. In *Biological basis of sex differences in psychopharmacology* (pp. 15-36): Springer.

Olivier, J. D., Blom, T., Arentsen, T., & Homberg, J. R. (2011). The age-dependent effects of selective serotonin reuptake inhibitors in humans and rodents: A review. *Prog Neuropsychopharmacol Biol Psychiatry, 35*(6), 1400-1408. doi:10.1016/j.pnpbp.2010.09.013

Olivier, J. D., Valles, A., van Heesch, F., Afrasiab-Middelman, A., Roelofs, J. J., Jonkers, M., . . . Homberg, J. R. (2011). Fluoxetine administration to pregnant rats increases anxiety-related behavior in the offspring. *Psychopharmacology (Berl), 217*(3), 419-432. doi:10.1007/s00213-011-2299-z

Pawluski, J. L., Li, M., & Lonstein, J. S. (2019). Serotonin and motherhood: From molecules to mood. *Front Neuroendocrinol*. doi:10.1016/j.yfrne.2019.03.001

Pfaff, D. W. (1999). *Drive: Neurobiological and molecular mechanisms of sexual motivation.*

Rayen, I., Steinbusch, H. W., Charlier, T. D., & Pawluski, J. L. (2014). Developmental fluoxetine exposure facilitates sexual behavior in female offspring. *Psychopharmacology, 231*(1), 123-133. doi: 10.1007/s00213-013-3215-5

Snoeren, E. M., Chan, J. S., de Jong, T. R., Waldinger, M. D., Olivier, B., & Oosting, R. S. (2011). A new female rat animal model for hypoactive sexual desire disorder; behavioral and pharmacological evidence. *J Sex Med, 8*(1), 44-56. doi:10.1111/j.1743-6109.2010.01998.x

Snoeren, E. M., Veening, J. G., Olivier, B., & Oosting, R. S. (2014a). Serotonin 1A receptors and sexual behavior in female rats: a review. *Pharmacology Biochemistry and Behavior, 121*, 43-52.

Snoeren, E. M., Veening, J. G., Olivier, B., & Oosting, R. S. (2014b). Serotonin 1A receptors and sexual behavior in male rats: a review. *Pharmacology Biochemistry and Behavior, 121*, 102-114.

Spiteri, T., Musatov, S., Ogawa, S., Ribeiro, A., Pfaff, D. W., & Ågmo, A. (2010). Estrogen-induced sexual incentive motivation, proceptivity and receptivity depend on a functional estrogen receptor α in the ventromedial nucleus of the hypothalamus but not in the amygdala. *Neuroendocrinology, 91*(2), 142-154.

Uphouse, L. (2000). Female gonadal hormones, serotonin, and sexual receptivity. *Brain Res Brain Res Rev, 33*(2-3), 242-257.

Uphouse, L. (2014). Pharmacology of serotonin and female sexual behavior. *Pharmacology Biochemistry and Behavior, 121*, 31-42. doi: 10.1016/j.pbb2013.11.008

Uphouse, L., & Guptarak, J. (2010). Serotonin and Sexual Behavior. In C. P. Müller & B. L. Jacobs (Eds.), *Handbook of the Behavioral Neurobiology of Serotonin* (Vol. 21, pp. 347-365): Elsevier. doi:10.1016/s1569-7339(10)70089-8

Walf, A. A., Rhodes, M. E., & Frye, C. A. (2006). Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. *Neurobiol Learn Mem, 86*(1), 35-46. doi:10.1016/j.nlm.2006.01.004

Appendix A

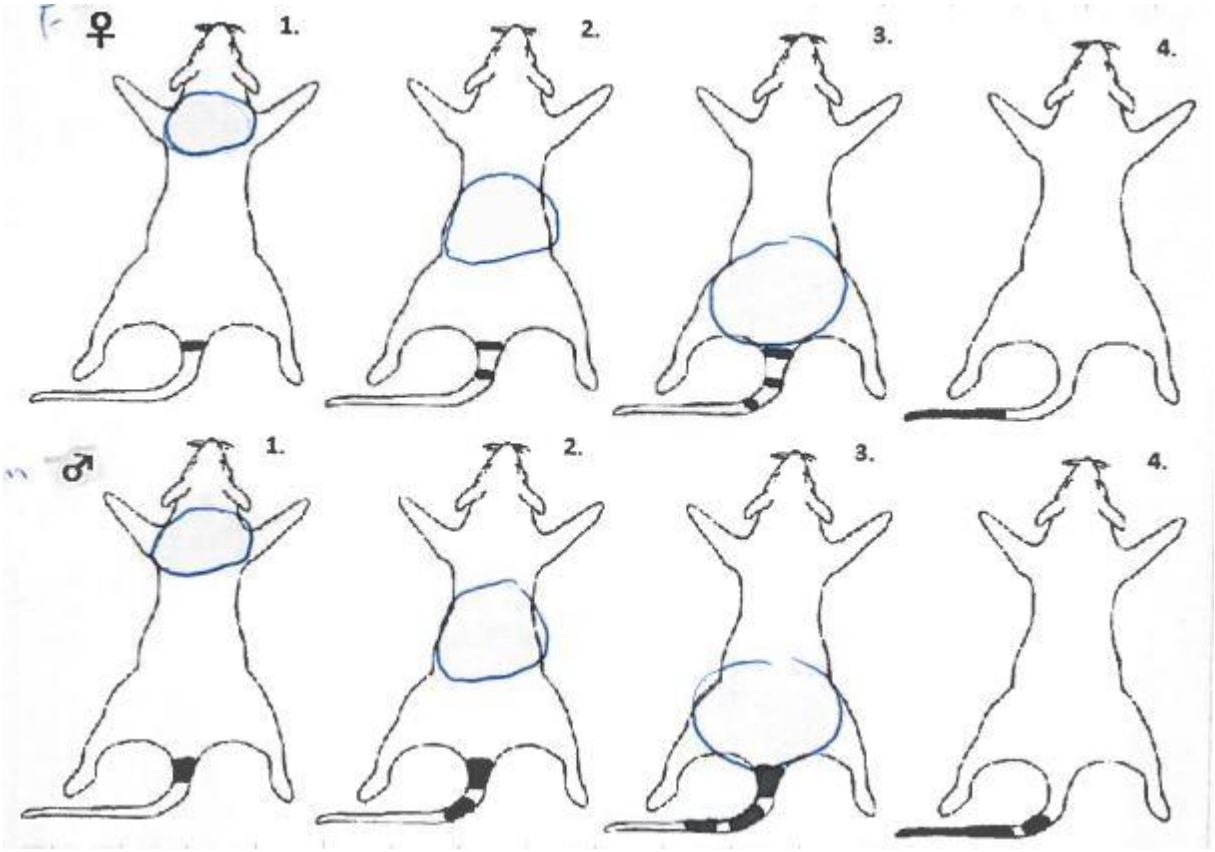


Figure A1. The blue circles indicate the area which was shaved in order to identify the different rats once they where in the seminatural environment. In addition to the shaved area the tails were also marked with stripes.

Appendix B

Table B1

Results of Mann-Whitney U tests of time spent or numbers of various behaviors during oestrous period. Data are mean ± SEM

Behavior	Ctrl females	Flx Females	Z	p
Boxing/wrestling	0.0162 ± 0.0048	0.0153 ± 0.0037	-0.105	.959
Nose-off	0.0080 ± 0.0033	0.0080 ± 0.0040	-0.105	.959
Rejection	0.0029 ± 0.0014	0.0017 ± 0.0008	-0.525	.626
Sniffing others	0.0495 ± 0.0183	0.0398 ± 0.0073	-0.525	.645
Grooming others	0.0008 ± 0.0005	0.0019 ± 0.0011	-0.893	.398
Anogenital sniffing	0.0043 ± 0.0021	0.0035 ± 0.0017	-0.525	.645
Paracopulatory	0.0353 ± 0.0092	0.0425 ± 0.0086	-0.735	.505
Lordosis	0.0053 ± 0.0011	0.0056 ± 0.0008	-0.315	.798
Mount	0.0032 ± 0.0007	0.0032 ± 0.0005	-0.210	.878
Intro	0.0014 ± 0.0004	0.0015 ± 0.0003	-0.210	.878
Ejaculation	0.0001 ± < 0.0000	0.0002 ± < 0.0000	-0.959	.374
LQ	0.0144 ± 0.0053	0.0093 ± 0.0028	-0.210	.878
Social behavior	0.0546 ± 0.0180	0.0453 ± 0.0088	-0.210	.878
Conflict behavior	0.0271 ± 0.0075	0.0251 ± 0.0061	-0.315	.798
Received copulation	0.0047 ± 0.0010	0.0049 ± 0.0007	-0.210	.878

Note. Z = values from the non-parametric test, *p* is the exact computed value.

Appendix C

Table C1

Results of Mann-Whitney U test of time spent or number of various behaviors for the most active bout. Data are mean ± SEM

Behavior	Ctrl females	Flx Females	Z	p
Boxing/wrestling	0.0147 ± 0.0040	0.0149 ± 0.0042	-0.210	.878
Nose-off	0.0070 ± 0.0035	0.0083 ± 0.0037	0.000	.999
Rejection	0.1493 ± 0.05	0.1246 ± 0.05	-0.368	.745
Sniffing others	0.0506 ± 0.0067	0.0366 ± 0.0066	-0.105	.959
Grooming others	0.0003 ± 0.0004	0.0008 ± 0.0004	-0.213	.876
Anogenital sniffing	0.0046 ± 0.0031	0.0042 ± 0.0026	-0.420	.721
Paracopulatory	0.0372 ± 0.0078	0.0399 ± 0.0075	-0.420	.721
Lordosis	0.0048 ± 0.0013	0.0056 ± 0.0009	-0.789	.457
Mount	0.0029 ± 0.0008	0.0030 ± 0.0004	-0.315	.777
Intro	0.0014 ± 0.0005	0.0018 ± 0.0005	-0.580	.591
Ejaculation	0.0002 ± 0.0001	0.0002 ± <0.0001	-1.217	.239
LQ	0.0262 ± 0.0089	0.0146 ± 0.0029	-0.315	.798
Social behavior	0.9254 ± 0.012	0.9174 ± 0.012	-0.315	.798
Conflict behavior	0.1711 ± 0.0544	0.1477 ± 0.0540	-0.210	.787
Received copulation	0.0045 ± 0.0013	0.0050 ± 0.0009	-0.736	.487

Note. Z = values from the non-parametric test, *p* is the exact computed value.

Appendix D

Table D1

Summary of Mann-Whitney U test of time spent or number of behaviors per timebin between Flx females and Ctrl females.

		Timebin																			
Behavior		5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Anogenital sniffing	Flx	3.70 ± 1.91	3.47 ± 2.12	3.93 ± 2.89	0.44 ± 0.34	3.00 ± 1.75	0.08 ±0.07	10.53 ± 8.40	1.67 ± 0.78	2.74 ± 1.27	1.74 ± 0.90	2.60 ± 2.28	0.30 ± 0.30	1.59 ± 1.10	2.00 ± 1.27	0.83 ± 0.83	7.45 ± 6.76	1.02 ± 0.67	2.00 ± 0.90	12.36 ± 6.36	2.23 ± 2.09
	Ctrl	0.22 ± 0.22	0.42 ± 0.31	0.17 ± 0.17	2.91 ± 2.68	9.52 ± 4.65	0.40 ± 0.29	0.92 ± 0.70	2.86 ± 1.96	3.20 ± 2.75	0.64 ± 0.36	1.22 ± 1.22	1.39 ± 0.74	0.35 ± 0.20	0.00	3.26 ± 2.91	0.78 ± 0.45	0.00	0.09 ± 0.09	0.44 ± 0.44	1.84 ± 1.37
	Z	-1.991	-1.852	-1.660	-0.511	-0.877	-0.770	-1.206	-0.483	-0.845	-0.543	-1.035	-1.173	-0.128	-2.207	-0.616	-0.302	-1.460	-0.770	-1.788	0.000
	sig	.063	.067	.128	.713	.420	.467	.249	.648	.478	.615	.446	.323	.999	.077	.733	.765	.467	.467	.077	.999
Sniffing other	Flx	33.80 ± 8.88	47.05 ± 23.34	21.60 ± 7.60	21.45 ± 6	21.58 ± 7.01	17.66 ± 5	29.56 ± 9.94	27.53 ± 10.23	52.64 ± 19.01	35.32 ± 16.59	27.90 ± 7.48	29.79 ± 9.36	28.23 ± 6.93	37.66 ± 18.73	29.06 ± 14.39	22.76 ± 6.81	33.82 ± 17.63	19.29 ± 5.51	17.21 ± 6.32	27.80 ± 9.75
	Ctrl	16.08 ± 9.71	20.07 ± 11.05	72.36 ± 66.50	77.44 ± 73.07	62.60 ± 48.79	35 ± 25.67	41.98 ± 36.75	62.60 ± 51.32	92.78 ± 83.81	31.33 ± 26.26	69.28 ± 54.16	79.48 ± 68.52	40.97 ± 34.57	28.23 ± 22.93	15.72 ± 8.54	13.86 ± 4.81	15.77 ± 10.02	22.18 ± 16.65	22.75 ± 14.28	24.31 ± 14.01
	Z	-1.904	-1.890	-0.735	-1.682	-0.105	-0.578	-1.164	-0.316	-1.580	-1.640	-0.946	-1.156	-1.685	-1.791	-1.369	-1.053	-1.890	-1.314	-0.735	-1.470
	sig	.058	.065	.505	.101	.933	.595	.260	.798	.129	.111	.367	.267	.104	.082	.194	.326	.065	.203	.505	.161
Social behavior	Flx	37.51 ± 9.78	**50.52 ± 23.85**	26.76 ± 9.92	26.47 ± 10.25	25.59 ± 8.35	17.92 ± 5.09	40.33 ± 17.56	31.55 ± 10.57	55 ± 38 ± 19.71	37.67 ± 17.46	31.24 ± 8.50	30.64 ± 9.29	29.82 ± 7.20	39.95 ± 20.20	31.04 ± 16.30	29.62 ± 11.03	34.85 ± 17.52	20.59 ± 6.08	29.57 ± 12.21	30.03 ± 9.68
	Ctrl	16.30 ± 9.73	**20.90 ± 11.39**	72.53 ± 66.48	80.35 ± 72.97	72.38 ± 47.82	35.40 ± 25.63	42.89 ± 36.66	65.87 ± 51.09	95.98 ± 83.43	43.60 ± 26.67	68.89 ± 54.42	80.87 ± 68.38	42.04 ± 34.43	28.43 ± 23.13	18.98 ± 10.68	14.64 ± 5.07	16.46 ± 9.92	22.27 ± 16.64	23.19 ± 14.25	26.15 ± 14.30
	Z	-1.904	**-2.102**	-1.050	-1.261	-0.210	-0.578	-1.164	-0.316	-1.369	-0.843	-1.159	-1.156	-1.475	-1.791	-1.369	-0.843	-1.890	-1.314	-0.840	-1.365
	sig	.058	**.035**	.328	.225	.853	.595	.260	.798	.194	.440	.277	.267	.159	.082	.194	.440	.065	.203	.442	.195
Conflict behavior	Flx	13.20 ± 4.48	13.47 ± 9.68	4.29 ± 1.82	10.77 ± 5.34	10.06 ± 2.86	5.02 ± 2.97	10.27 ± 3.23	50.05 ± 22.77	49.93 ± 18.66	56.05 ± 30.43	28.20 ± 12.26	41.52 ± 22.73	34.27 ± 13.56	23.97 ± 12.04	29.10 ± 10.81	14.67 ± 6.03	13.63 ± 6.65	20.44 ± 7.03	22.64 ± 17.27	29.21 ± 13.81
	Ctrl	51.05 ± 38.57	23.33 ± 13.57	19.58 ± 9.72	32.15 ± 22.32	15.88 ± 9.33	7.66 ± 4.38	22.16 ± 18.77	12.33 ± 6.11	40.02 ± 36.76	7.28 ± 2.95	3.82 ± 1.44	30.72 ± 20.98	32.66 ± 24.73	18.02 ± 11.30	10.45 ± 5.95	11.39 ± 6.35	10.84 ± 6.97	12.97 ± 8.89	24.37 ± 19.89	12.20 ± 4.53
	Z	-0.053	-0.0684	-0.701	-0.216	-0.316	-0.158	-0.987	-1.386	-1.534	-1.279	-1.706	-1.270	-1.685	-1.217	-1.425	-0.527	-0.640	-1.708	-0.329	-0.635
	sig	.973	.521	.508	.846	.798	.900	.360	.188	.137	.226	.099	.218	.104	.244	.175	.624	.566	.308	.788	.555
Paracopulatory behavior	Flx	44.51 ± 14.33	19.81 ± 10.72	14.04 ± 5.75	15.63 ± 5.67	59.44 ± 27.89	65.39 ± 28.44	36.42 ± 16.21	43.66 ± 20.78	33.03 ± 15.31	32.24 ± 12.34	21.25 ± 7.82	17.97 ± 8.76	8.73 ± 5.51	31.55 ± 11.86	41.62 ± 14.15	15.85 ± 11.71	16.88 ± 8.52	15.63 ± 8.43	6.71 ± 3.61	21.16 ± 13.24
	Ctrl	27.72 ± 9.99	91.30 ± 77.65	41.75 ± 36.08	11.79 ± 6.64	5.95 ± 3.49	8.82 ± 3.75	15.86 ± 7.29	7.40 ± 3.49	19.74 ± 14.52	21.65 ± 19.62	10.66 ± 8.27	3.15 ± 2.39	5.17 ± 2.58	32.50 ± 30.10	2.76 ± 1.57	7.58 ± 2.87	40.07 ± 22.17	10.98 ± 6.25	6.82 ± 4.01	25.25 ± 17.88
	Z	-1.104	-0.737	-0.317	-0.755	-1.864	-1.348	-0.767	-1.386	-0.270	-1.186	-1.754	-1.504	-0.505	-1.510	-2.426	-0.219	-0.162	-0.054	0.000	0.000
	sig	.292	.504	.782	.479	.070	.187	.482	.188	.821	.259	.088	.158	.634	.145	.015	.875	.905	.988	.999	.999
Recieved copulation	Flx	1.86 ± 0.64	2 ± 1.24	1 ± 0.33	0.75 ± 0.62	4.88 ± 3.50	8.376 ± 3.62	5.63 ± 2.18	**5.25 ± 2.18**	5.75 ± 2.47	6.75 ± 2.10	4.38 ± 1.61	4.75 ± 2.40	3.50 ± 2.43	4.13 ± 1.74	5.13 ± 1.86	6 ± ± 3.18	3.38 ± 2.10	1.38 ± 0.73	0.63 ± 0.63	**6.88 ± 1.89**
	Ctrl	3.25 ± 1.06	7.63 ± 6.24	3.38 ± 2.55	2 ± 1.13	0.50 ± 0.27	1.63 ± 1	2.75 ± 1.50	**0.5 ± 0.5**	3.63 ± 1.94	2.50 ± 1.39	2.88 ± 1.60	2.38 ± 1.25	1.50 ± 0.98	3.25 ± 2.42	1.88 ± 1.09	2.13 ± 1.20	5.13 ± 3.68	2.50 ± 0.94	1 ± 0.63	**1.38 ± 1.10**
	Z	-0.966	-0.405	-0111	-0.766	-1.302	-1.890	-1.067	**-2.493**	-0.603	-1.836	-0.787	-1.098	-0.725	-1.191	-1.296	-1.010	-0.406	-0.824	-0.967	**-2.031**
	sig	.371	.745	.924	.426	.210	.063	.315	**.018**	.579	.069	.476	.294	.543	.249	.218	.341	.765	.429	.569	**.047**
Lordosis	Flx	3.75 ± 1.08	2.13 ± 1.09	1.25 ± 0.31	1.88 ± 0.81	6.25 ± 4.17	8.75 ± 3.74	5.88 ± 2.37	**6.13 ± 1.79**	6.25 ± 2.74	**7 ± 2.19**	4.63 ± 1.73	4.13 ± 1.86	3.75 ± 2.68	4.13 ± 1.74	5.63 ± 2.06	3 ± 1.43	3.5 ± 2.19	1.75 ± 0.84	0.75 ± 0.62	**8.13 ± 2.26**
	Ctrl	4.13 ± 1.23	8.38 ± 6.43	3 ± 2.18	1.88 ± 1.04	0.88 ± 0.61	1.63 ± 1.12	3 ± 1.57	**0.50 ± 0.38**	3.75 ± 2.04	**1.75 ± 1.37**	3.75 ± 2.18	2.28 ± 1.16	1.38 ± 0.91	3.25 ± 2.42	2.13 ± 1.23	1.75 ± 1.03	5.50 ± 3.78	2.50 ± 0.94	0.88 ± 0.61	**1.88 ± 1.03**
	Z	-0.161	-0.548	-0.494	-0.232	-1.605	-2.052	-0.878	**-2.414**	-0.659	**-2.194**	-0.674	-1.098	-0.785	-1.191	-1.296	-0.844	-0.405	-0.488	-0.449	**-2.414**
	sig	.901	.632	.687	.902	.130	.042	.403	**.018**	.554	**.029**	.532	.294	.510	.249	.218	.439	.745	.667	.928	**.019**

Note. Bold indicates significant differences between the two compared groups. $N = 8$ in each group.
Data are mean ± SEM, z values from the Mann-Whitney U test, exact p – values reported.