UiT The Arctic University of Norway

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The Effects of Perinatal Fluoxetine Exposure on Social and Non-Social Investigation Behaviors in a Novel Environment

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Preface

From early in the program, we have both preferred the more tangible sides of psychology and wanted to conduct our thesis within the field of neuroscience. Beforehand, we had some experience with animal research from the semester thesis, and we aimed to do something similar for our main thesis.

The journey began the autumn 2018 when we contacted the research group *Behavioral and Translational Neuroscience* at University of Tromsø. The group leader Associate Professor Eelke Snoeren and Postdoc Indrek Heinla introduced us to an ambitious project on the behavioral effects of perinatal antidepressant exposure in rats. The project demanded high motivation and effort from us, but in return we received top-class supervision and an invitation to join their research group. We are very grateful for the opportunity to experience an academic environment from the inside.

We received training in a behavioral scoring software, and after some practice we started scoring rat behavior from pre-existing video material. We collected data during the summer break of 2019 and the following semester. After finishing the data collection, we spent a significant amount of time getting familiar with, structuring, cleaning and running statistical analysis on the data. Throughout the project, we have worked independently on the various tasks from data collection to writing.

During our thesis project we have learned a lot about the scientific process and experienced the persistency it takes to conduct real research. We have worked hard and are proud of our final product.

Finally, we would like to thank our main supervisor Dr. Eelke Snoeren and co-supervisor Dr. Indrek Heinla for their supervision, support and engagement in the project. We are also grateful to the other members of the research group for including us and for sharing their knowledge with us. In addition, we would like to thank our families and friends for their support.

Tromsø, 7th of July 2020.

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) are increasingly prescribed as medication for various affective disorders during pregnancy. SSRIs cross the placenta and affect serotonergic neurotransmission in the fetus, but the neurobehavioral consequences for the offspring remain largely unclear. Recent research has linked perinatal SSRI exposure to alterations in both social and non-social aspects of behavior. However, this research has mainly focused on behavior within simplified environments. The current study investigates the effects of perinatal exposure to SSRIs on social and non-social investigation behaviors when the individual is introduced to a novel seminatural environment with unfamiliar conspecifics. During the perinatal period (gestational day 1 until postnatal day 21), rat dams received daily either SSRI treatment (fluoxetine 10 mg/kg) or vehicle. Four cohorts of offspring, each consisting of four males and four females, were observed in adulthood during the first hour within a seminatural environment. The results showed that perinatal fluoxetine exposure altered aspects of non-social, but not social, investigation behaviors. Both fluoxetine exposed male and female rats spent more time on walking/running than controls, while fluoxetine exposed females also walked/ran more often than control animals. Furthermore, compared to control, fluoxetine exposed female rats spent less time exploring objects and specific elements in the physical environment. We suggest that perinatal exposure to SSRI lead to a quicker, less detailed investigation strategy in novel environments, and that the alteration is most pronounced in females. Whether this effect is disadvantageous or not is yet to be revealed.

Keywords: antidepressants, fluoxetine, perinatal, social behavior, non-social behavior, rats, seminatural environment, SSRI

The Effects of Perinatal Fluoxetine Exposure on Social and Non-Social Investigation Behaviors in a Novel Environment

A considerable number of women experience depression or other mental disorders during pregnancy. Approximately 1 of 10 pregnant women fulfill the DSM-5 diagnostic criteria for major depressive disorder (Bennett, Einarson, Taddio, Koren, & Einarson, 2004; Woody, Ferrari, Siskind, Whiteford, & Harris, 2017). In treatment of maternal depression and anxiety, selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed class of drugs. The prescription rate of SSRIs to pregnant women has increased dramatically in the last decades (Mitchell et al., 2011), and recent estimates suggest a worldwide prevalence of 3% (Molenaar et al., 2020) with significant geographically differences (Andrade et al., 2008; Charlton et al., 2015). That is, hundreds of thousands of babies exposed to SSRIs during early development are born every year. Despite the widespread use, we have limited knowledge on whether SSRI exposure during the early stages of brain development can lead to adverse long-term outcomes, such as alterations in social and non-social behaviors.

Antidepressants, such as SSRIs, reaches the fetus by crossing the placenta and are also present in the breast milk (Kristensen et al., 1999; Rampono, Proud, Hackett, Kristensen, & Ilett, 2004). Thus, children can potentially be exposed to SSRI during the whole perinatal period (Kim et al., 2006; Noorlander et al., 2008). SSRIs works by inhibiting the function of the serotonin transporter (SERT or 5-HTT), which is responsible for the reuptake of serotonin (5-HT) at the plasma membrane. Inhibition of 5-HTT leads to an accumulation of 5-HT in the extracellular space. This in turn increases the magnitude and duration of 5-HT activity at pre- and post-synaptic 5-HT receptors. In the adult brain, 5-HT acts mainly as a modulatory neurotransmitter, regulating emotion, cognition, sleep and stress responses (Olivier, Blom, Arentsen, & Homberg, 2011). However, in the early developing brain, 5-HT is widespread and acts as a neurotropic factor regulating cell division, differentiation, migration, and synaptogenesis (Azmitia, 2001; Gaspar, Cases, & Maroteaux, 2003). Thus, alterations in serotonergic functioning during early stages of development may have long-lasting effects on the offspring.

Since 5-HT plays an important role in neurodevelopment (Glover & Clinton, 2016), developmental SSRI exposure is suggested to affect brain maturation and behavior (Muller, Anacker, & Veenstra-VanderWeele, 2016). However, the clinical literature on neurobehavioral outcomes is scarce and inconclusive. Most of the literature has examined the childhood years, and only a few studies on early adolescence or beyond exists (Malm et al., 2016). Thus, potential long-term consequences into adulthood are largely unknown. However, findings in children show associations between developmental SSRI exposure and socio-emotional, behavioral and cognitive outcomes. There is found a link to impaired social behavior (Klinger et al., 2011), an increased risk of speech and language disorders (Brown et al., 2016), and elevated levels of internalizing behavior, like anxiety and depression (Hermansen, Røysamb, Augusti, & Melinder, 2016; Lupattelli et al., 2018; Malm et al., 2016).

Recently, a possible link between developmental SSRI exposure and neurodevelopmental disorders has been examined. A meta-analysis by Halvorsen, Hesel, Østergaard and Danielsen (2019) revealed that in utero SSRI exposure was associated with later diagnosis of autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD) and intellectual disability in children. Nevertheless, the possible vulnerability to ASD is a subject of controversy. Serotonergic (dys)functioning is hypothesized to play a role in ASD pathogenesis due to 5-HTs role in social behavior and multiple neurodevelopmental processes (Glover & Clinton, 2016; Muller et al., 2016). However, the correlation between developmental SSRI exposure and ASD tend to disappear when controlled for maternal mood and stress (Brown et al., 2016; Clements et al., 2015). Confounding factors such as severity of maternal depression or shared genetical predisposition may explain the association between SSRI exposure and adverse outcomes (Glover & Clinton, 2016; Ornoy, 2017).

Epidemiological research on humans, like the above-mentioned studies, are correlational of nature, and do not necessarily imply causation. A frequent problem with human studies is the difficulty to isolate the effects of SSRI exposure from the effects of maternal mental health. Women using SSRI during pregnancy are likely suffering from depression which itself has been shown to have negative impact on the offspring (Dunkel Schetter, 2011; El Marroun, White, Verhulst, & Tiemeier, 2014; Goodman, 2007). Animal research, on the other hand, allows to control for potential interference from confounding factors, like maternal health, drug dose and timing of exposure. By using randomized controlled experiments, animal studies can provide fundamental insight into the underlying mechanisms of perinatal SSRI exposure. Furthermore, as rodent and human serotonergic development is remarkably similar (Glover & Clinton, 2016), rodent studies can provide valuable translational insight about how developmental SSRI exposure affect human offspring.

Animal studies investigating the effects of developmental exposure to SSRI have reported alterations in different social and non-social behaviors in the offspring. In juvenile male and female rats, both pre- and post-natal SSRI exposure have been shown to decrease social play behavior in offspring (Houwing, Staal, et al., 2019; Khatri, Simpson, Lin, & Paul, 2014; Olivier, Vallès, et al., 2011; Rodriguez-Porcel et al., 2011; Simpson et al., 2011). Similar tendencies have been found in adult rats with developmental SSRI exposure leading to less social interactions (Olivier, Vallès, et al., 2011; Rodriguez-Porcel et al., 2011), or decreased interest to explore a novel conspecific (Khatri et al., 2014; Rodriguez-Porcel et al., 2011; Simpson et al., 2011; Zimmerberg & Germeyan, 2015). SSRI exposure can also increase aggressive-like social behaviors in adult male mice (Gemmel et al., 2017; Kiryanova & Dyck, 2014; Svirsky, Levy, & Avitsur, 2016). Furthermore, some studies have reported reduced activity and explorative behaviors in perinatal SSRI exposed adult rats and mice (Karpova, Lindholm, Pruunsild, Timmusk, & Castrén, 2009; Lee & Lee, 2012; Lisboa, Oliveira, Costa, Venâncio, & Moreira, 2007; Ramsteijn et al., 2020; Rebello et al., 2014; Sarkar, Chachra, & Vaidya, 2014).

Unfortunately, most of the animal studies have used simplified rodent test set-ups which only investigates a small fraction of all behaviors. Furthermore, these studies do not account for the environmental and social complexity of real-world situations. To bypass this limitation, recent studies from our research group have employed a seminatural environment enabling rats to express all aspects of their natural behaviors (Hegstad et al., 2020; Heinla, Heijkoop, Houwing, Olivier, & Snoeren, 2020; Houwing, Heijkoop, Olivier, & Snoeren, 2019). These studies showed that perinatal SSRI fluoxetine (FLX) exposure lead to various alterations in social and non-social behaviors in a naturalistic setting. More specifically, perinatal fluoxetine exposure increased the amount of passive social behaviors in both males and females, but reduced active social behavior, general activity and pro-social behaviors in females. After a white-noise stressor, FLX-females shifted from resting more socially to resting proportionally more alone, while the FLX-males increased the amount of self-grooming, and freezing in the open area. Interestingly, these studies were performed in the seminatural environment *after* the rats were familiarized to each other and the physical environment. It is currently unknown how social and non-social behaviors manifest when the animals get into a novel environment with unfamiliar conspecifics. Since handling new and unknown situations is such an important part of everyday life, we are interested to know whether perinatal SSRI exposure affect behaviors when introduced to unfamiliarity.

The aim of the current study was to investigate if perinatal SSRI exposure alters social and non-social investigation behaviors in a novel environment with unknown conspecifics. We define investigation as behaviors that, within a novel situation, provides the animal with information about a stimulus. More specifically, social investigation refers to when the stimulus investigated is another rat, such as when sniffing and grooming others, while non-social investigation refers to investigation of physical objects and environmental locations. Fluoxetine or vehicle was administered to pregnant female rats from gestational day 1 until the pups were weaned at postnatal day (PND) 21. Since neurodevelopment in rats at PND 1-10 equals the third semester of pregnancy in humans (Andrews & Fitzgerald, 1997; Dobbing & Sands, 1979), this timeframe simulates the entire human pregnancy period, and part of the postnatal period. Cohorts of eight offspring were housed in a seminatural environment and observed during the first hour after introduction. In line with previous studies (Heinla et al., 2020; Houwing, Heijkoop, et al., 2019), we expect perinatal fluoxetine exposure to alter both social and non-social investigation behaviors in the initial phase of the introduction to the seminatural environment. A recently published meta-analysis (Ramsteijn et al., 2020) found indication of sex-effects on behavioral outcomes in antidepressant-exposed offspring. In line with these findings, this study will treat males and females separately.

Material and Methods

This experiment is based on video material from another study (Houwing, Heijkoop, et al., 2019). Since pre-existing video recordings were employed, the authors of the current study were not involved in the video collection, but rather started the project at the stage of observation. Thus, the animals and procedures in our study were similar as described in (Hegstad et al., 2020; Heinla et al., 2020; Houwing, Heijkoop, et al., 2019). However, the behavioral scoring scheme was uniquely formed to the current study. The following section will describe all relevant experimentation steps.

Animals and Dam Housing

As potential parents of the offspring, a total of 20 Wistar rats (10 males, 10 females), weighting 200-250 grams on arrival, were obtained from Charles River (Sulzfeld, Germany) for breeding. After arrival, the rats were placed in same-sex pairs into Makrolon® IV cages (60 x 38 x 20 cm) on a reversed 12:12 hours light/dark cycle, in which the lights were turned on at 23.00. To ensure thermal comfort, the room had temperatures of 21 ± 1°C, and relative humidity of 55 ± 10%. Standard rodent food pellets (standard chow, Special Diets Services, Witham, Essex, UK), water and nesting material were available ad libitum. Animal care and experimental procedures were conducted in agreement with European Union council directive 2010/63/EU. The protocol was approved by the National Animal Research Authority in Norway.

Breeding and Antidepressant Treatment

Daily, all females were checked for sexual receptivity by placing them together with a male rat for 5 minutes. When lordosis behavior was observed, they were considered in proestrus and thus ready for breeding. The female then got placed together with a male in an isolated Makrolon® IV cage for the next 24 hours (gestational day 0). Afterwards, they returned to their initial same-sex pairs for the first two weeks of pregnancy. From gestational day 14, the females were placed alone until delivery (gestational day 21/postnatal day 0).

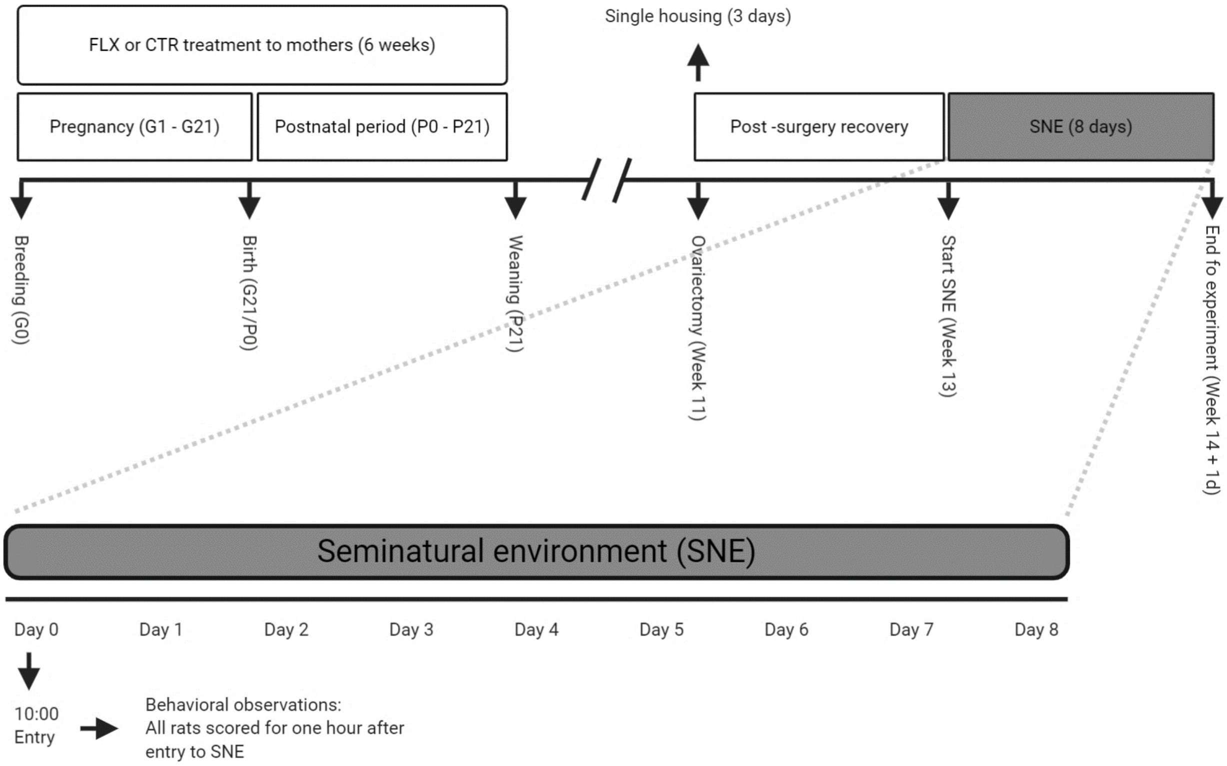
During a 6-week period, from conception (gestational day 0) to weaning (postnatal day 21), females were administered daily with either SSRI fluoxetine 10 mg/kg (Apotekproduksjon, Oslo, Norway) or vehicle (methylcellulose (Sigma, St. Louis, MO, USA)) per oral gavage. The offspring were thus exposed to perinatal fluoxetine via the treatment of the dams (in uterus and via breast feeding). The fluoxetine treatment was prepared with tablets for human usage that were pulverized and dissolved in sterile water (2mg/mL) and injected at a volume of 5mL/kg. Methylcellulose powder, the non-active filling of a fluoxetine tablet, was used as control condition. The powder was dissolved in sterile water to create a 1% solution and administered at a volume of 5mL/kg as well. Every third day, females were weighted to ensure correct dosage of fluoxetine/vehicle. The chosen dosage of fluoxetine was decided upon comparison of fluoxetine blood levels of humans and animals (Lundmark, Reis, & Bengtsson, 2001; Olivier et al., 2011). When the rat dams got close to the end of pregnancy, they were checked two times a day (09.00 and 15.00) for delivery.

Offspring Housing

The offspring were housed together with their mothers until weaning (gestational day 21). After weaning, groups of two or three same-sex littermates were housed together in Makrolon® IV cages (see cage distribution in Appendix A). They were left undisturbed, except for the ovariectomy (see section for Procedure) and weekly cage cleaning, until introduction to the seminatural environment at the age of 13-18 weeks. To enable individual recognition, ears were punched. In Figure 1, a schematic overview shows all experimental procedures from gestational day 0 to the end of the experiment.

Figure 1

Overview of Experimental Procedures



Note. FLX = fluoxetine, CTR = control, P = postnatal day, G = gestational day.

Seminatural Environment

The seminatural environment (SNE; 240 x 210 x 75 cm) consisted of two parts: an open area and a burrow system (Figure 2; Chu & Ågmo, 2014; Houwing, Heijkoop, et al., 2019; Snoeren et al., 2015). Four openings (8 x 8 cm) connected the two areas. In the open area, two partitions (40 x 75 cm) simulated natural obstacles. The burrow system consisted of connected tunnels (width 7.6 cm, height 8 cm) and four nest boxes (20 x 20 x 20 cm). Plexiglas covered the burrow at the height of 75 cm, while the open area remained open. A curtain between the two places allowed for different light settings. The burrow was left dark the whole time. In the open area, on the other hand, light settings simulated a day-night cycle. A lamp, located 250 cm above the floor, did simulate day light (180 lux) between 22.45 and

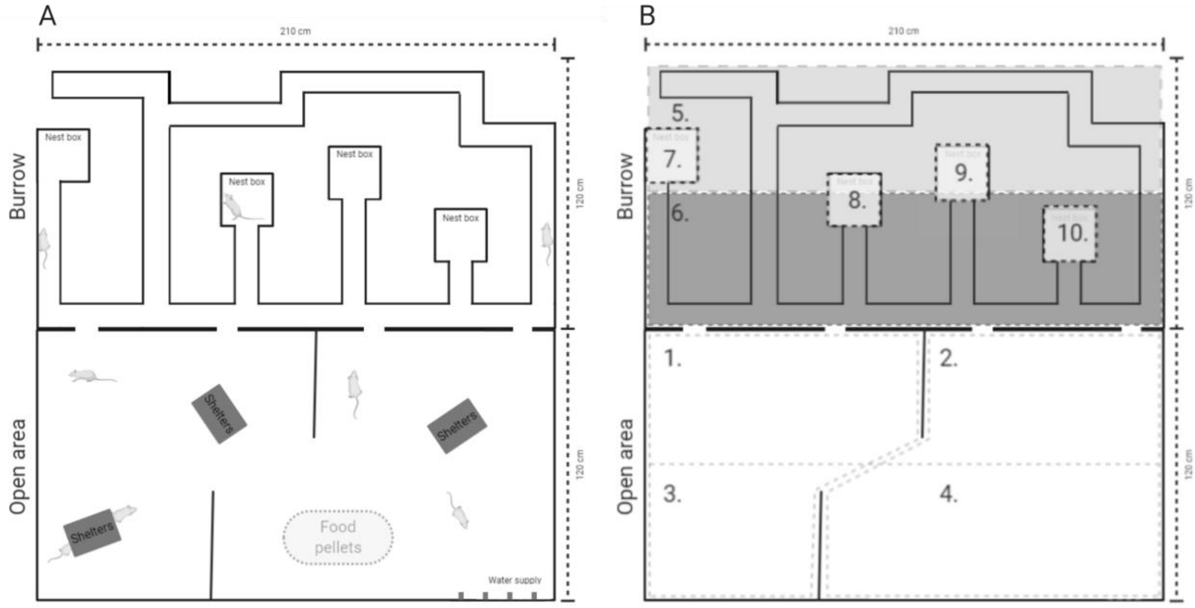
10.30. From 10.30 to 11.00 the lights gradually decreased to 1 lux (simulating moonlight). Similarly, the light increased gradually from 1 to 180 lux between 22.15 and 22.45.

The whole ground of the SNE was covered with a layer (2 cm) of aspen wood chip bedding (Tapvei, Harjumaa, Estonia). The nest boxes had 6 squares of nesting material in each (non-woven hemp fibers, 5 x 5 fibers, 5 mm thickness, Datesend, Manchester, UK). Three plastic shelters (15 x 16.5 x 8.5 cm, Datesend, Manchester, UK) were placed in the open area. Additionally, 12 aspen wooden sticks (2 x 2 x 10 cm, Tapvei, Harjumaa, Estonia) were randomly placed around in the SNE. A pile of food pellets (approx. 2 kg) and four bottles of water were available at all time (see location in Figure 2A).

Two video cameras (©Basler) were mounted on the ceiling, respectively 2 m above the open area (regular camera) and the burrow system (infrared camera). Media Recorder 2.5 was employed for video recordings. The data got immediately stored on an external hard drive. The recording was manually stopped and restarted every 24 hours. The purpose was to ensure that eventual errors only would affect one day of recorded data.

Figure 2

The Seminatural Environment



Note. Illustration of the whole seminatural environment (A) and sectioning of the different locations (B). 1 = OA close to burrow left, 2 = OA close to burrow right, 3 = OA far away from burrow left, 4 = OA far away from burrow right, 5 = tunnels far away from OA, 6 = tunnels close to OA, 7 = nestbox left, 8 = nestbox mid-left, 9 = nestbox mid-right, 10 = nestbox right.

Design of the Study

Initially, five cohorts, each consisting of eight rat offspring, were placed one at the time in the SNE. However, one day of video material was lost due to recording error, which in turn reduced the number of cohorts to four. A cohort consisted of 4 males and 4 females whereof each sex constituted 2 controls (CTR) and 2 fluoxetine (FLX) rats. Thus, data from this experiment came from 8 CTR males, 8 CTR females, 8 FLX males and 8 FLX females. Within a cohort, same sex rats came from different litters and were thus unfamiliar to each other. Some rats had one sibling from the opposite sex in the same cohort. However, these rats were housed in different home cages since weaning.

Procedure

For the purpose of a previous study (Houwing, Heijkoop, et al., 2019), the female offspring were ovariectomized two weeks before entering the SNE in order to control their estrous cycle. Although irrelevant for the objective of the current study, this procedure had the effect of keeping the females in diestrus of the menstrual cycle during the observation period. Before entering the SNE, the rats were shaved on the back and tail-marked under isoflurane anesthesia for individual recognition (for more details, see (Houwing, Heijkoop, et al., 2019)). All rats were also weighted, confirming that there was no weight difference between CTR- and FLX-rats.

Each cohort was placed in the SNE for 8 days. See Figure 1 for an overview of the whole procedure. The cohorts were introduced to SNE on the first day (day 0) at 10.00 and removed on day 8 at the same time. However, only data from the first day was used for the purpose of this study. All rats were again weighted after being removed from the SNE. No difference in weight was observed between CTR- and FLX-rats. In order to remove olfactory clues, the SNE was cleaned between cohorts.

Behavioral Observations

The frequency and/or duration of several behaviors (see Table 1) were scored manually by using Observer XT, version 12 (Noldus, Wageningen, The Netherlands). Two observers, blinded for the animal treatment, separately scored either males or females across all four cohorts. In addition to behavior, (1) location of the animal (see Figure 2B), (2) whether the animal initiated or was respondent to the respective behavior, (3) whether the animal was in physical contact with another animal or not during the respective behavior, and lastly, (4) ID of the eventual interacting partner was scored. Since we were interested in observing how the rats encountered a novel environment with unfamiliar conspecifics, all rats were scored in the first 60 minutes after entry to the SNE.

Table 1

Description of Recorded Behaviors

Behavior	Description
Walking/running	Walking or running through the environment
Chasing	Running forward in the direction of a conspecific
Non-social exploration	Exploring the environment by sniffing, usually when slowly walking or sitting still
Digging	Digging, pushing or carrying bedding/nesting/food material
Resting/immobile alone	Sitting or sleeping with minimal movement of the head without other rats in close vicinity
Resting/immobile socially	Sitting or sleeping with minimal movement of the head with at least 1 other rat on maximum 1 rat body length away
Hiding alone	Being in the shelter alone
Hiding socially	Being in the shelter with at least one other rat
Following	Walking or running in the same direction as another rat in front.
Allogrooming	Grooming any part of the partners body, usually on the head or in the neck region
Sniffing anogenitally	Sniffing the anogenital region of the conspecific
Sniffing nose-to-nose	Sniffing the facial region of the conspecific
Sniffing body	Sniffing any part of the conspecifics body, except for the anogenital and facial region
Fighting	Kicking, pouncing, pushing, grapping, boxing or wrestling another rat
Nose-off	Facing another rat, usually in a tunnel, resulting in one rat moving forward and the other backing up
Self-grooming	Grooming itself
Freezing	Complete absence of movement in addition to a tense body posture
Rearing supported	Raising itself upright on its hind pawn toward a wall or an object
Rearing unsupported	Raising itself upright on its hind pawn not toward a wall or an object

Table 2

Description of Behavioral Clusters

Cluster	Behaviors within clusters
Socially active behaviors	Combines sniffing anogenitally, sniffing nose-to-nose, sniffing body, and allogrooming
General activity	Combines walking/running, and non-social exploration
Non-socially passive behaviors	Combines resting alone, hiding alone
Socially passive behaviors	Combines hiding socially, and resting socially
Conflict behaviors	Combines nose-off, and fighting

Data Preparation and Statistical Analysis

As shown in Table 2, behavioral clusters were made from the recorded behaviors. For each rat, we calculated the total duration and the number of events for every behavior and behavioral cluster. This data was later divided into six 10-minute timebins in order to analyze behavioral changes over time. Latencies to meet the other rats, and latencies to visit the different locations of the SNE was also noted. This data was later divided and analyzed over 1/3/5/10/20/30/60 minutes. In this study, we operationalized social investigation behaviors as the cluster “socially active behaviors” and the latencies to meet all other rats, whereas non-social investigation behaviors were operationalized as the cluster “general activity” and latencies to visit all the locations (See Figure 2B).

Normality of data was determined with Shapiro-Wilks tests. Data with $p < .05$ was analyzed non-parametrically. Simple group comparisons were performed with either a student t-test or the non-parametric Mann-Whitney U test. Repeated measures ANOVA was used when the behaviors were analyzed over time. In cases the Mauchly’s test indicated violation of sphericity from the ANOVA output, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. To correct for multiple comparisons, the Benjamini-Hochberg procedure was performed on all significant results together with a predetermined set of variables (sniffing, self-grooming, non-social exploration, conflict behaviors). All p-values are reported after the Benjamini-Hochberg correction (original p-values can be found in Appendix B). All tests reported were done 2-sided.

Results

From the behavioral scoring, we obtained a lot of data. Both total duration and frequency of all behaviors performed during the first hour was noted. This considered, we cannot discuss all behaviors separately in this result section. An overview of all behaviors can be found in Appendix B to D. In the following section, the most relevant and interesting findings from the experiment will be presented.

Fluoxetine Exposure Does Not Influence Social Behaviors in a Novel Environment

We first asked whether perinatal exposure to fluoxetine (FLX) affect social behaviors when the animals are placed in a new environment. Previous research has shown that perinatal FLX-exposure might affect social behaviors in a naturalistic setting (Heinla et al., 2020; Houwing, Heijkoop, et al., 2019), but it is unknown how introduction to a novel environment with unknown conspecifics might affect social behaviors in perinatally FLX-exposed rats. We therefore examined social investigation behaviors, as in how the rats investigate unknown conspecifics. In addition, we measured other relevant forms of social behaviors during the first period after introduction.

Social Investigation Behaviors

The data analysis revealed that CTR- and FLX-females did not differ in time spent on (t = -1.04, p = .315, d = -0.52, Figure 3A) or number of episodes (t = -1.04, p = .318, d = -0.52) performing socially active behaviors. When looking separately at the different behavioral components constituting the cluster (see Table 2), a trend indicated that FLX-females spent more time sniffing anogenitally compared to CTR-females (t = -2.13, p = .051, d = - 1.07). However, CTR- and FLX-rats did not differ on any other behavioral components constituting the clusters relevant to social behaviors (socially active behaviors, socially passive behaviors and conflict behaviors). No difference was found between CTR- and FLX-males for socially active behaviors in total time (t = 0.95, p = .356, d = 0.48, Figure 3D) or on number of episodes (t = 0.103, p = .919, d = 0.05).

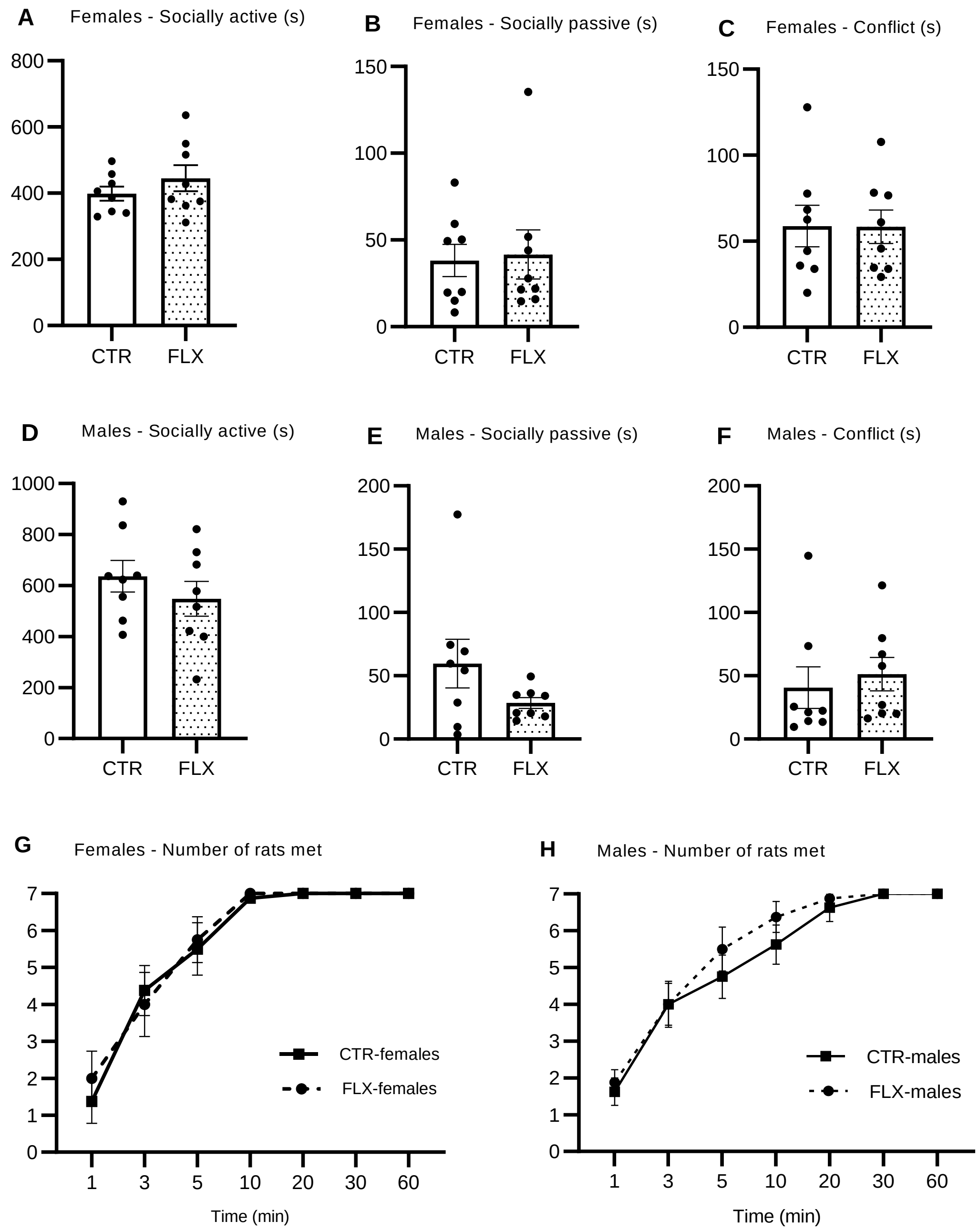
Although the treatment groups did not differ in the amount of socially active behaviors, it could still be the case that the groups had different interest in meeting other rats. To investigate this possibility, we first looked at the latencies to when the rats had met all seven other cohort-members. The data analysis showed that there was no significant difference between CTR- and FLX-rats for neither females (*t* = 0.84, *p* = .418, *d* = 0.42) nor males (*U* = 24.00, *z* = -0.84, *p* = .422, *r* = -.21) in latency to meet all cohort-members. We thereafter measured how many cohort members the rats had met as a function of time. CTR- and FLX-rats were compared on cumulative data measured at 1/3/5/10/20/30/60 minutes. For FLX-females, there were no significant differences in the number of rats met (treatment effect: *F*(1,14) = 0.05, *p* = .821) or in the pattern of rats met (timepoints x treatment: *F*(1.73, 24.24) = 0.28, *p* = .725) over time compared to CTR-females (Figure 3G). Similarly, CTR- and FLX-males did not differ in the number of rats met across all timepoints (treatment effect: *F*(1,14) = 0.49, *p* = .492) or in the pattern of rat met over time (timepoints x treatment: *F*(2.05, 28.74) = 0.59, *p* = .563, Figure 3H).

Other Social Behaviors

We also investigated some other social behaviors such as socially passive behaviors and conflict behaviors. No difference was found between CTR- and FLX-females in total time (*U* = 33.00, *z* = 1.05, *p* = 1, *r* = .03, Figure 3B) or number of episodes being socially passive (*t* = -0.28, *p* = .784, *d* = -0.14). Furthermore, CTR- and FLX-females spent similar amount of time (*t* = 0.03, *p* = .978, *d* = 0.01, Figure 3C) and episodes (*t* = -0.40, *p* = .692, *d* = -0.20) in conflict with other rats. Similarly, for males, no differences were found for time spent on social passive behavior (*U* = 41.00, *z* = 0.95, *p* = .382, *r* = .24, Figure 3E), episodes of social passive behavior (*t* = 1.48, *p* = .161, *d* = 0.74), time spent on conflict behavior (*t* = -0.03, *p* = .655, *d* = -0.02, Figure 3F), or episodes in conflict behavior (*U* = , 42.00, *z* = 1.05 *p* = .786, *r* = .26).

Figure 3

Social Behaviors in Females and Males



Note. The data represent the time spent (s) on socially active behaviors (A, D), socially passive behaviors (B, E), conflict behaviors (C, F), and the total number of rats met over time (G, H). All graphs show comparisons between CTR-females (n = 8) and FLX-females (n = 8) or between CTR-males (n = 8) and FLX-males (n = 8). Data are shown with individual data points, with bars representing the mean ± SEM.

Fluoxetine Exposure Influence How the Animals Investigate a Novel Environment

The results thus far suggest that perinatal exposure to fluoxetine does not lead to alterations in social behaviors when animals are placed in a novel environment with unknown conspecifics. We next investigated whether perinatal fluoxetine exposure alter non-social investigation behaviors, as in how the animals investigate objects and the physical environment. We also examined other relevant non-social behaviors during the first period after introduction to the environment.

Non-Social Investigation Behaviors

CTR- and FLX-females did not differ on time spent on (t = -1.04, p = .311, d = 0.31, Figure 4A) or on the number of episodes in general activity (t = -1.82, p = .090, d = -.0.91). However, when looking more in detail, FLX-females were found to spend significant more time walking/running (U = 56.00, z = 2.52, p = .025, r = .63, Figure 4B; this and further significant p-values are corrected with the Benjamini-Hochberg procedure, all original values can be found in Appendix B) but *less* time on non-social exploration (U = 8.00, z = -2.52, p = .025, r = - .63, Figure 4C) compared to CTR-females. FLX-females were also found to walk/run more often than CTR-females (t = -4.29, p = .005, d = -2.15). CTR- and FLX-females did not differ in the number of non-social exploration episodes (t = -0.54, p = .693, d = -0.27). Similar as for the females, no difference in time spent on (t = -1.69, p = .114, d = -0.85, Figure 4D) or on number of episodes in general activity (t = -1.60, p = 0.131, d = -0.80) were found between CTR- and FLX-males. However, FLX-males spent more time walking/running than CTR-males (t = -3.05, p = .045, d = -1.52, Figure 4E), but there was no difference in time spent on non-social exploration (t = 0.06, p = .953, d = 0.03, Figure 4F). FLX-males did not differ from CTR-males on the number of episodes walking/running (t = -1.61, p = .130, d = -0.80) or on non-social exploration (t = -0.73, p = .786, d = -0.36).

We then investigated whether there were differences between CTR- and FLX-rats in how long it took to visit all the 10 locations (see Figure 2B) of the seminatural environment. Rats that did not visit all locations within the observation time were given a score of 3600 (total observation time in seconds). The results indicated that FLX-rats, both males and females, did not spend significant more time to visit all locations than CTR-rats (females: t = 1.33 = p = .212, d = 0.42; males: t = -1.15, p = .271, d = -0.57). We thereafter investigated how many locations the rats visited as a function of time (1/3/5/10/20/30/60 minutes). A trend indicated that FLX-females were faster at visiting the different locations (treatment effect: F(1,14) = 4.15, p = .061; time x treatment: F(2.92, 40.94) = 2.41, p = .081, Figure 4G). A post-hoc analysis revealed that FLX-females visited significant more locations within the first 3 minutes (t = -2.46, p = .027, d = -1.23) compared to CTR-females. No difference in the number of locations visited (treatment effect: F(1,14) = 3.43, p = .085) or in the pattern (time x treatment: F(2.64, 36.97) = 0.39, p = .735) over time were found between the CTR- and FLX-males (Figure 4H).

Other Non-Social Behaviors

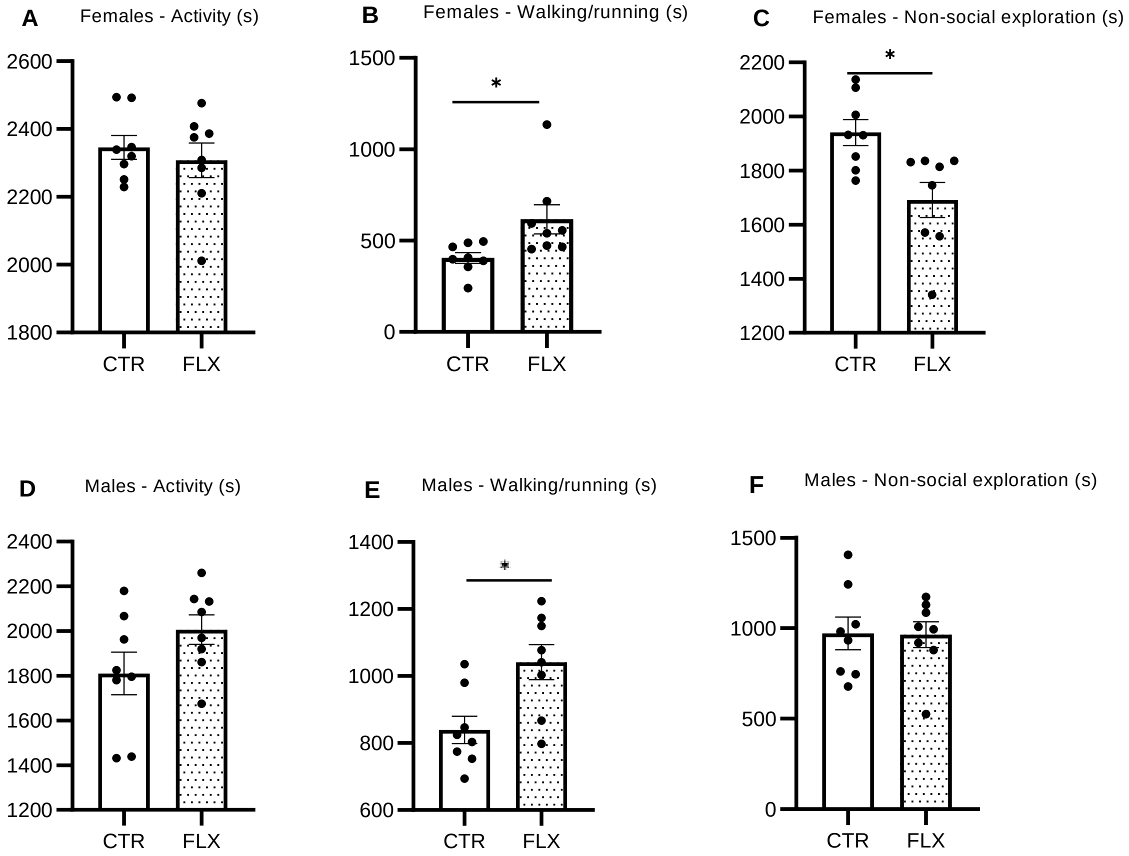
We also compared CTR- and FLX-rats on other relevant non-social behaviors, including non-socially passive and stress-coping behaviors. The analysis revealed that there was no significant difference between CTR- and FLX-females in time spent on (U = 28.00, z = -0.42, p = .721, r = -0.11) or in the number of non-socially passive behaviors (t = -0.12, p = .903, d = -0.06). Similarly, for the male groups, no significant difference was found for time spent on (t = 1.62, p = .127, d = 0.81) or in the number of non-socially passive behaviors (t = 0.62, p = .546, d = 0.31)

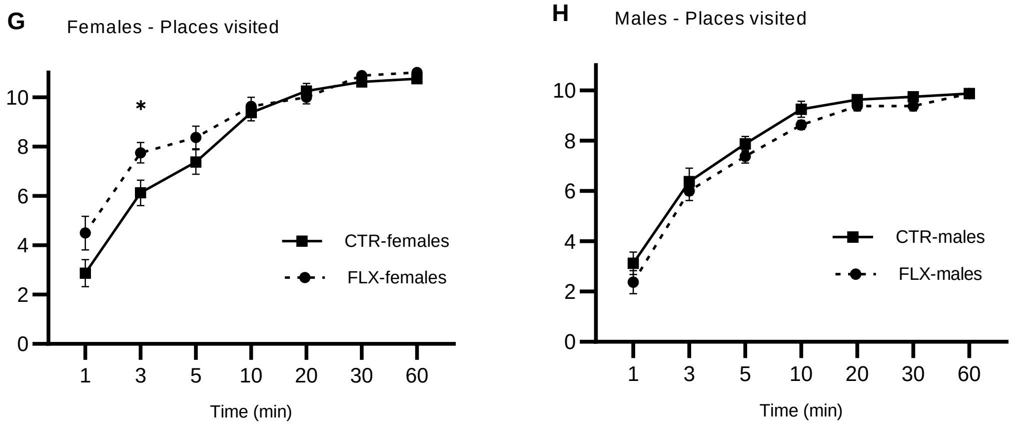
Next, we investigated whether CTR- and FLX-rats showed different level of stress-coping behaviors. The results revealed no significant difference between CTR- and FLX-rats for time spent on (females: t = 1.67, p = .195, d = 0.84; males: U = 37.00, z = 0.53, p = .806, r

= .13) or in the number of episodes (females: *t* = 0.58, *p* = .693, *d* = 0.29; males: *t* = -0.60, *p* = .860, *d* = -0.30) self-grooming. Neither did the treatment groups differ on time spent on (females: *t* = - 0.70, *p* = .497, *d* = - 0.35; males: *t* = -0.61, *p* = .554, *d* = -0.30) or number of episodes (females: *t* = -1.23, *p* = .240, *d* = -0.61; males: *t* = -0.48, *p* = .638, *d* = -0.24) rearing unsupported. When investigating the total time in the open area, no significant difference was found between CTR- and FLX-rats (females: *t* = -1.39, *p* = .186, *d* = -0.70; males: *t* = -0.98, *p* = .345, *d* = -0.49). Similarly, the treatment groups did not differ on the total time spent in the burrow area (females: *t* = 1.57, *p* = .138, *d* = 0.79; males: *t* = 1.02, *p* = .323, *d* = 0.51).

Figure 4

Non-Social Behaviors in Females and Males





Note. The data represent the time spent (s) on general activity (A, D), walking/running (B, E), non-social exploration (C, F), and the total number of places in the SNE visited over time (G, H). All graphs show comparisons between CTR-females (n = 8) and FLX-females (n = 8) or between CTR-males (n = 8) and FLX-males (n = 8). Data are shown with individual data points, with bars representing the mean ± SEM.

$^*p < 0.05$

Fluoxetine Exposure Does Not Influence How the Rats Adapt to a Novel Environment

Finally, we were interested to see whether the treatment groups adapted differently to the novel physical and social environment, and thus, whether the differences in behavior between the groups were stable over time. We therefore divided the dataset into six 10-minutes timebins and assessed the differences between CTR- and FLX-rats on social and non-social behaviors over the course of the observation period. (See Appendix C for time effects).

Social Investigation Behaviors

The repeated measure analysis revealed that FLX-females and FLX-males did not show a significantly different pattern of time spent on socially active behaviors, compared to CTR-females (timebin x treatment: *F*(5,70) = 0.26, *p* = .932, η_p^2 = .02, Figure 5A) or CTR-males (timebin x treatment: *F*(5,70) = 0.51, *p* = .765, η_p^2 = .04, Figure 5B) respectively. Similarly, when looking at the frequency of socially active behaviors, no interaction between timebin and treatment was found for female ($F(5,70) = 0.63$, $p = .675$, $\eta_p^2 = .04$) or male ($F(5,70) = 0.99$, $p = .431$, $\eta_p^2 = .07$) rats.

Non-Social Investigation Behaviors

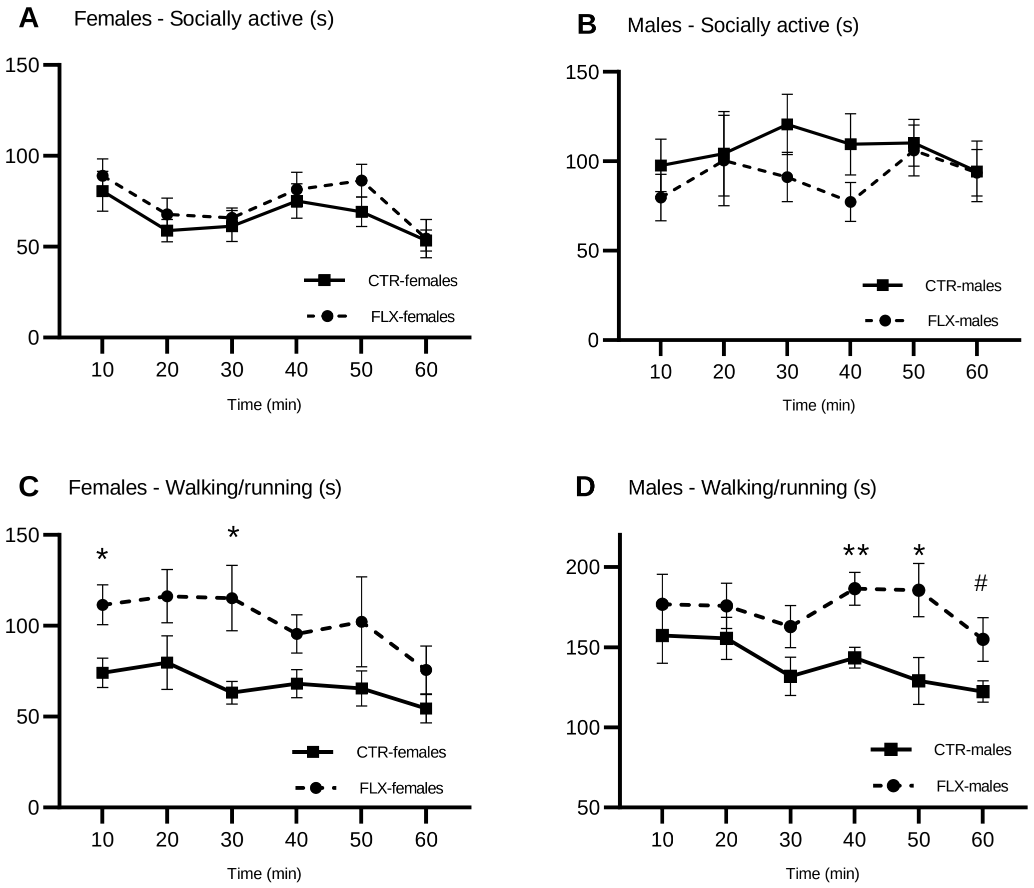
For time spent on walking/running, no differences as a function of time were found between the CTR- and FLX-rats for females ($F(5,70) = 0.63$, $p = .679$, $\eta_p^2 = .04$) or males ($F(2.64. 36.92) = 0.69$, $p = .634$, $\eta_p^2 = .05$). Similar results were found when analyzing the frequency of walking/running (females: $F(5,70) = 0.88$, $p = .498$, $\eta_p^2 = .06$; males: $F(2.85, 39.88) = 0.82$, $p = .483$, $\eta_p^2 = .06$). In term of non-social exploration, neither FLX-females ($F(5,70) = 0.84$, $p = .529$, $\eta_p^2 = .06$) nor FLX-males ($F(2.87, 40.20) = 0.47$, $p = .697$, $\eta_p^2 = .03$) showed a significant different pattern of time spent on exploration compared to their control group. Similar results were revealed for the frequency of non-social exploration (females: $F(5,70) = 0.23$, $p = .948$, $\eta_p^2 = .02$; males: $F(5,70) = 1.76$, $p = .132$, $\eta_p^2 = .11$).

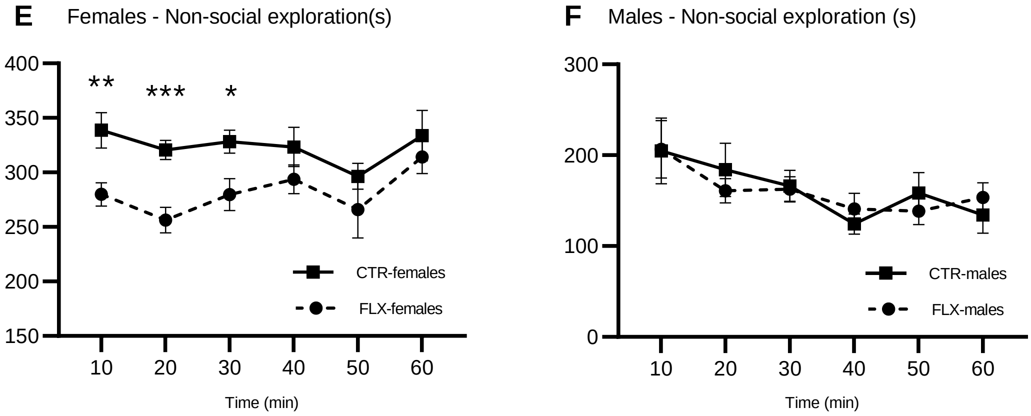
Other Social and Non-Social Behaviors

CTR- and FLX-rats showed no significant difference in pattern of time spent on (females: timebin x treatment: $F(1.21, 16.87) = 0.17$, $p = .729$, $\eta_p^2 = .01$; males: timebin x treatment: $F(1.89, 26.42) = 1.05$, $p = .361$, $\eta_p^2 = .07$) and number of (females: timebin x treatment: : $F(1.72, 24,12) = 0.41$, $p = .636$, $\eta_p^2 = .03$; males: timebin x treatment: $F(2.04, 28.58) = 1.01$, $p = .378$, $\eta_p^2 = .07$) socially passive behaviors. Neither did they show any significant difference in pattern of time spent on (females: timebin x treatment: $F(5,70) = 1.49$, $p = .204$, $\eta_p^2 = .10$; males: timebin x treatment: $F(1.99, 27.88) = 0.98$, $p = .388$, $\eta_p^2 = .07$) or number of (females: timebin x treatment: $F(5,70) = 0.63$, $p = .680$, $\eta_p^2 = .04$; males: timebin x treatment: $F(1.92, 26.84) = 0.77$, $p = .468$, $\eta_p^2 = .05$) conflict behaviors. However, when analyzing the data non-linearly, a cubic interaction effect for the females group indicated that when one group scored higher, the other scored lower ($F(1, 14) = 5.71$, $p = .031$, $\eta_p^2 = .29$). For non-socially passive behaviors, no significant difference as a function of time were found for females (duration: timebin x treatment: $F(2.23, 31.14) = 0.26$, $p = .795$, $\eta_p^2 = .02$; frequency: timebin x treatment: $F(2.64, 36.99) = 0.18$, $p = .889$, $\eta_p^2 = .01$) and males (duration: timebin x treatment: $F(2.33, 32.62) = 0.58$, $p = .592$, $\eta_p^2 = .04$; frequency: timebin x treatment: $F(2.56, 35.90) = 0.51$, $p = .649$, $\eta_p^2 = .04$).

Figure 5

Social and Non-Social Investigation Behaviors Measured as a Function of Time.





Note. The data represent the time spent (s) on different behaviors as a function of time measured every 10 minutes. The graphs show socially active behaviors (A, B), walking/running (C, D), and non-social exploration (E, F). All graphs show comparisons between CTR-females (n = 8) and FLX-females (n = 8) or between CTR-males (n = 8) and FLX-males (n = 8). Data are shown with individual data points, with bars representing the mean ± SEM.

***p < 0.001, ** p < 0.01, *p < 0.05, #p < 0.06

Discussion

In our study, we investigated how perinatal fluoxetine exposure affect social and non-social investigation behaviors in a novel seminatural environment at adulthood. Our findings show that fluoxetine exposure does not induce alterations on social investigation behaviors. However, fluoxetine exposure was found to affect non-social investigation behaviors. More specifically, fluoxetine exposed female and male rats showed an increased tendency to walk/run, while fluoxetine exposed females showed a decreased tendency to non-social exploration. Furthermore, it was demonstrated that fluoxetine exposure does not affect how the animals adapt to a novel environment with unfamiliar conspecifics over time.

Social Behaviors

The first question we asked was whether social investigation behaviors, operationalized as active social behaviors (sniffing and grooming other rats) and latency to meet all other colony members, would be affected by perinatal SSRI exposure. The ability to interact in line with social norms is crucial in everyday life, and deviant social behavior in the initial phase of contact can make it difficult to establish social relationships. The results in this study revealed no difference between CTR- and FLX-rats on the total time spent on, or the number of, active social behaviors. Contrary to our present results, a recent study from our research group showed that FLX-females, but not FLX-males, showed a tendency toward decreased active social behaviors (Houwing, Heijkoop, et al., 2019). However, in another study (Heinla et al., 2020), no difference was found between CTR- and FLX-rats on active social behaviors, in line with the present results. Nevertheless, in those studies, behaviors were observed after the rats had already been housed together in the seminatural environment for several days, and thus were familiar with each other. The effect of fluoxetine on social behaviors might have different outcomes depending on whether the rats are interacting with familiar or unfamiliar partners (Gemmel et al., 2019). In the present study, the rats were observed during the first hour after introduction to the seminatural environment, allowing us to investigate how the rats encounter the first social situations before knowing each other.

We also looked at how long it took the rats to meet the other colony members after being introduced to the novel environment. Such latency times could indicate whether the rats have different interests in approaching other rats. Lack of social interest is a relevant trait to examine since such symptoms commonly appears in various mental and neurodevelopmental disorders (Barkus & Badcock, 2019). The results did however not reveal any difference in latency between CTR- and FLX-rats. From our findings, we conclude that perinatal SSRI exposure does not affect social investigation behavior during the first hour after introduction to a novel environment.

We further investigated whether SSRI exposure leads to behavioral alterations in other aspects of social behaviors, such as social passive behaviors and conflict behaviors. Contrary to the findings of Houwing, Heijkoop, et al (2019), in which perinatal FLX exposure caused an increase in passive social behavior, our data showed no differences in this behavior. Furthermore, neither FLX-females nor FLX-males differed from CTR-rats in terms of conflict behavior. However, conflict behavior was not frequently occurring in our experiment. The Wistar strain is generally known to exhibit little aggressive behavior compared to other strains (Koolhaas et al., 2013). In addition, the experiment was not designed to trigger aggressive behavior as competition for food, water or mating partners were not necessary.

In total, the results indicate that perinatal SSRI exposure does not affect social behaviors when introduced to unfamiliar conspecifics. However, as another study found altered social behaviors in FLX-rats several days after introduction to the seminatural environment (Houwing, Heijkoop, et al., 2019), we suggest that the effect of SSRI exposure on social behaviors might be dependent on the degree of familiarity between the rats.

Non-Social Behaviors

Next, we asked whether perinatal SSRI exposure would affect non-social investigation behaviors, operationalized as walking/running, non-social exploration and latency to visit all locations of the environment. Investigatory behaviors towards objects and physical surroundings are frequently occurring in freely moving rats, and therefore makes up robust variables. These behaviors are also important in everyday life, and potential SSRI-induced effects on non-social investigation behaviors could possibly impact people's quality of life.

In the current study, we found that both FLX-females and FLX-males spent more time walking/running compared to control rats. FLX-females also had a higher frequency of episodes with walking or running compared to CTR-females. In addition, FLX-females visited more locations of the seminatural environment within the first 3 minutes after entrance compared to CTR-females. Together, this could indicate that perinatal SSRI exposure leads to an increased interest to investigate paths and locations. Contrary to our findings, a recent meta-analysis found evidence for *reduced* activity in developmentally SSRI exposed rats, as mostly measured by total distance moved (Ramsteijn et al., 2020). Although we did not measure total distance per se, it is reasonable to assume that the distance is related to total time spent walking/running. Anyway, the meta-analysis is mainly based on studies measuring activity in simplified open field boxes. Such set-ups allow the rats to see the whole environment without necessarily having to move their bodies. We could therefore assume that an increased interest to investigate locations and paths would only be observable in situations where walking/running (movement) is needed to investigate the environment. Hence, increased walking/running would only appear in complex set-ups such as the seminatural environment. In addition, given that the increase in walking/running reflects an alteration in interest to investigate novel paths and locations, we would expect the differences between FLX- and CTR-rats to disappear (or diminish) when the animals get familiar with their surroundings. Interestingly, previous studies from our research group, employing data from the same experiment, found no differences on walking/running between FLX- and CTR- rats after the rats were already familiarized with the environment (Heinla et al., 2020; Houwing, Heijkoop, et al., 2019). Thus, the complexity of the environment can not isolated explain the increased walking/running in our study. Rather, the complex environment must be unfamiliar to the rat in order to induce increased walking/running. Taken together, perinatal SSRI seems to increase the interest to investigate locations and paths only when exposed to a novel complex environment.

Furthermore, we also found that FLX-females, but not FLX-males, spent less time on non-social exploration than control rats, meaning they were sniffing less on objects (e.g. shelters, wooden sticks) and specific elements in the physical environment (e.g. walls, the ground). This is in line with previous findings from day 4 and day 7 in the same experiment (Houwing, Heijkoop, et al., 2019), where reduced non-social exploration was found in FLX-females, but not in FLX-males. Other studies have also reported reduced non-social explorative behaviors in SSRI exposed rats (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004; Karpova et al., 2009; Rebello et al., 2014; Sarkar et al., 2014; Simpson et al., 2011; Zohar, Shoham, & Weinstock, 2016). Although we have earlier shown that FLX-females seem to have increased interest to explore paths and locations, this current finding indicates that perinatal SSRI exposure in females leads to reduced interest to investigate objects and other specific elements in the environment. Although the findings might seem contradictive at first sight, walking/running and non-social exploration could possibly serve different purposes. The increased walking/running could reflect a tendency to screen the environment more quickly, as opposed to detailed and accurate investigation as reflected by non-social exploration. Therefore, we suggest that perinatal SSRI exposure alters the strategy the animals use to investigate a novel environment, and that the alteration is most pronounced in females.

We further investigated other non-social behaviors such as stress-coping. We did not find any difference between CTR- and FLX-rats on stress-coping behaviors. A previous study found that white-noise exposure induced increased self-grooming in FLX-males (Houwing, Heijkoop, et al., 2019). As introduction to a new environment can be considered a stress-full situation, we expected to observe increased self-grooming in FLX-males also in the present study. However, as problematized in a previous paper (Houwing, Heijkoop, et al., 2019), self-grooming can serve several purposes, and thus be difficult to interpret. Unique grooming-patterns are identified for high- and low-stress situations (Smolinsky, Bergner, LaPorte, & Kalueff, 2009). Unfortunately, our video resolution does not allow to determine the grooming patterns in detail. Therefore, the value of this variable can be questioned. However, from our findings we conclude that SSRI exposure does not affect stress-coping behavior during the first hour.

In summary, contrary to previous studies, our findings suggest that perinatal SSRI exposure increases the tendency to walk/run. The data further indicate that SSRI exposed females are faster at checking out the unknown environment. Moreover, in line with previous studies, it was demonstrated that SSRI exposure reduce the tendency to perform non-social exploration in females. Taken together, we suggest that perinatal exposure to SSRI lead to a quicker, less detailed investigation strategy in a novel environment, and that the alteration is most pronounced in females. However, when translating to the human situation, it is difficult to determine whether these effects will be advantageous or disadvantageous.

Behavioral Adaption Over Time

The last question we asked was whether SSRI exposed rats adapt differently to unfamiliarity (both environmental and socially) than their non-exposed conspecifics. Therefore, we did split the observational data into six 10-minute timebins in order to look at behavioral changes over time. As part of the familiarization process to a new environment, we generally expected to see adjustments in behavior during the first hour (Wilkinson, Herrman, Palmatier, & Bevins, 2006). However, our main subject of interest was whether SSRI exposed rats adjusted their behavior in a different manner than controls.

Our results revealed that SSRI exposed animals adapted similarly to the novel environment as control animals. As discussed, FLX-females spent less time exploring objects and the physical environment, whereas both FLX-males and FLX-females spent more time walking/running compared to CTR-rats. Those differences remained relatively stable throughout the first hour, meaning that FLX- and CTR- rats behaved differently, but adapted similarly to the novel environment over time. However, when comparing the groups on isolated timebins across the first hour, FLX-females were non-socially exploring significantly less than controls only during the first 30 minutes (0-10, 10-20, 20-30 min). Interestingly, FLX-females walked/ran more than CTR-females between 0-10 and 20-30 min, but FLX-males on the other hand walked/ran more than controls between 30-40 and 40-50 min. Our experiment employed a reversed dark/light cycle in which the light conditions gradually decreased from daylight to moonlight between 10.30 and 11.00 every morning. The rats entered the seminatural environment for the first time at 10.00, and thereby experienced the shift from light to darkness during the first hour. Even though we found no significant treatment x time effects, the comparison of isolated timebins yield some interesting points for further discussion. FLX-females ran/walked more than controls in two timebins *before* 30 minutes (onset of light decrease), while FLX-males walked/ran more than controls in two timebins *after* 30 minutes. Moreover, the same pattern was present in non-social exploration in FLX-females. Thus, we started questioning whether FLX-rats are differently affected by changes in light conditions. In terms of walking/running, FLX-females and FLX-males behaved opposite of each other when compared to same-sex controls. Hence, the findings might indicate that SSRI exposure can lead to sex-specific alterations on behavioral adjustment to light.

Taken together, the data indicate that fluoxetine exposed rats do not adapt their behaviors differently than controls during the first hour after introduction to the novel environment. However, the effects of SSRI exposure on non-social investigation behaviors might be influenced by the environmental light conditions. For future research, it would be interesting to further examine the contribution of environmental light conditions on behavioral adaption.

Translational Value

Ultimately, experimentation on animals should be executed only when significantly contributing to advances in knowledge, and benefits for humans. Because our study is translational of nature, we want to highlight some aspects increasing the translational value of the current study.

First and foremost, we employed a seminatural environment. To ensure validity outside the laboratory, it has been argued to use a representative design constituted by elements simulating a natural habitat or real-word scenario (Brunswik, 1955). The seminatural environment aim to mimic features from the rat's natural environment and allows the rat to express its whole repertoire of behaviors. Because the animals are not constrained to a certain task or simple test-environment, the design is ideal to explore possible neurobehavioral effects. For translational purposes, it is typically of interest to examine all behaviors which mimics a human endophenotype (Crawley, 2012). The seminatural environment enables the researcher to examine several behavioral traits and relate them. For instance, in the case of autism spectrum disorder, the seminatural environment approach makes it possible to explore social interactions, olfactory communication, and repetitive behaviors such as self-grooming and digging, all within the same experiment. Furthermore, the seminatural environment allow to investigate whether such "symptoms" cause significant distress or impairment in functioning. In summary, we consider our design to have considerable translational advantageous in comparison to traditional test set-ups.

Conducting clinical research on the effects of perinatal SSRI exposure is both ethical and practical challenging. We therefore need both animal and human studies to complement each other in order to gain new insight. Developmental patterns of the serotonin system are remarkably similar in rodents and humans (Gingrich et al., 2017; Glover & Clinton, 2016). Therefore, rats are well-suited laboratory animals for investigating drugs that targets the serotonin transporter. In addition, we employed an outbred rat strain (Wistar rat) assuring high genetic variability which thus better resemble the human population (Cools, Brachten, Heeren, Willemen, & Ellenbroek, 1990).

We also enhanced the translational value by investigating both males and females separately. The serotonergic system matures differently in the female and male brain

(Brummelte, Mc Glanaghy, Bonnin, & Oberlander, 2017). The pre-clinical literature investigating both sexes is scarce, but indicates an effect of sex on behavioral outcomes after perinatal SSRI exposure (Ramsteijn et al., 2020). In the current study we found evidence for slightly different behavioral outcomes in males and females when introduced to a novel environment. The sex-effect should motivate future clinical researchers to study whether early SSRI exposure in humans is associated with different phenotypes in females and males at adulthood. So far, very limited research on effects of developmental SSRI exposure exists beyond the childhood-years (Malm et al., 2016).

Limitations

During the behavioral coding, both observers noted simultaneous and conspicuous freezing behavior across all rats in several cohorts. Retrospectively, we concluded that the freezing was triggered by noise from construction work (MH2, Campus Breivika, University of Tromsø). Since freezing behavior occurred in temporal context with an external stressor, the authors of the study collectively agreed on excluding freezing from further analysis.

In our study, we investigated the effects of perinatal SSRI exposure in offspring of healthy mothers. We could therefore isolate the effects of SSRIs from those possibly attributed to maternal stress/disorder. However, by employing healthy mothers we lost relatedness to the clinical situation. Pregnant women only take antidepressants if a psychiatric disorder is, or has been, present. Arguably, an animal model of depression, or another relevant psychopathology, would have mimicked the human situation better, and thus increased the translational value of the research.

Conclusion

In summary, we conclude that perinatal SSRI exposure alter aspects of non-social, but not social, investigation behaviors, when introduced to a novel environment with unfamiliar conspecifics. Both FLX-males and FLX-females showed a higher amount of walking and running, while FLX-females visited more locations within the first three minutes, and spent less time exploring objects and specific elements in the physical environment. Fluoxetine exposure did not affect how the animals adapted to the unfamiliar seminatural environment over time. We suggest that perinatal SSRI exposure alters non-social investigation, to a quicker and less detailed strategy, when exposed to a novel environment, and that the alteration is most pronounced in females. However, whether the altered investigation strategy is unfavorable or not remains to be revealed.

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Appendix A

Information on Dams and Offspring

Table A1

Treatment Dams and Cage Distribution Offspring

Dams	Treatment	Number offspring	Male-Cage 1	Male-Cage 2	Female-Cage 3	Female-Cage 4	Female-Cage 5	Female-Cage 6
F1	FLX	7	OM1 OM2 OM3		OF2 OF3	OF1 OF4		
F2	FLX	9	OM5 OM6 OM7	OM4 OM8	OF5 OF8	OF6 OF7		
F3	CTR	13	OM9 OM12	OM10 OM11	OF12 OF16	OF9 OF15	OF11 OF14	OF10 OF13 OF17
F4	CTR	6	OM13 OM14 OM15		OF18 OF19 OF20			
F5	FLX	None						
F6	FLX	None						
F7	CTR	None						
F8	CTR	15	OM16 OM17	OM20 OM21 OM25	OF21 OF23	OF22 OF24 OF25		
F9	FLX	8	OM29 OM30 OM31		OF27 OF29	OF26 OF28 OF30		
F10	FLX	dead						

Note. M = male, F= female, CTR = methylcellulose, FLX = fluoxetine, OM = male offspring, OF = female offspring

Table A2

Experimental Design

Colony	Male 1	Male 2	Male 3	Male 4	Female 1	Female 2	Female 3	Female 4
SNE1	OM1	OM5	OM9	OM13	OF2	OF5	OF12	OF21
SNE2	OM2	OM6	OM12	OM16	OF3	OF8	OF13	OF22
SNE3	OM3	OM7	OM15	OM17	OF1	OF6	OF15	OF24
SNE4	OM4	OM11	OM21	OM29	OF4	OF9	OF19	OF27
SNE5	OM8	OM10	OM20	OM30	OF7	OF14	OF20	OF26

Note. SNE = seminatural environment, OM = male offspring, OF female offspring

Appendix B

Results From Mann-Whitney-U-Test and t-Test

Table B1

Results From Mann-Whitney U-Test and t-Test on Duration of Behaviors

Behavior	Rats	Mean	SEM	Z	t	P
Socially active behaviors	CTR-females	398.55	21.30			
	FLX-females	445.08	39.20		-1.04	.315
	CTR-males	636.48	62.06			
	FLX-males	547.97	68.81		0.96	.356
General activity	CTR-females	2346.04	35.14			
	FLX-females	2307.96	51.21		0.61	.550
	CTR-males	1810.28	95.35			
	FLX-males	2006.00	66.08		-1.69	.114
Non-socially passive behaviors	CTR-females	568.14	59.66			
	FLX-females	614.69	80.48	- 0.42		.721
	CTR-males	273.71	44.14			
	FLX-males	163.04	30.57		1.62	.129
Socially passive behaviors	CTR-females	38.14	9.30			
	FLX-females	41.63	14.17	0.11		1.00
	CTR-males	29.15	10.04			
	FLX-males	29.56	6.67		-0.04	.973
Conflict behaviors	CTR-females	58.83	12.02			
	FLX-females	58.40	9.76		0.03	.978 (.978)
	CTR-males	40.60	16.52			
	FLX-males	51.19	13.19	0.95		.655 (.382)
Walking/running	**CTR-females**	**404.91**	**29.44**			
	FLX-females	**616.47**	**80.06**	**2.52**		**.025 (.010)**
	CTR-males	**839.01**	**40.73**			
	FLX-males	**1041.60**	**52.62**		**-3.05**	**.045 (.009)**
Chasing	CTR-females	0.34	0.34			
	FLX-females	0.00	0.00	-1.00		.721
	CTR-males	3.00	2.03			
	FLX-males	5.27	3.17	0.58		.645
Non-social exploration	**CTR-females**	**1941.13**	**48.02**			
	FLX-females	**1691.49**	**64.71**	**9.52**		**.025 (.010)**
	CTR-males	971.27	89.49			
	FLX-males	964.41	71.99		0.06	.953 (.953)
Digging	CTR-females	111.79	21.90			
	FLX-females	133.05	18.27		-0.75	.468
	CTR-males	34.17	14.37			
	FLX-males	33.88	14.59	0.00		1.00
Resting/immobile alone	CTR-females	21.80	14.68			
	FLX-females	24.77	9.15	0.79		.442
	CTR-males	183.84	35.40			
	FLX-males	116.07	27.60		1.51	.153
Resting/immobile socially	CTR-females	1.05	0.57			
	FLX-females	7.94	4.46	1.54		.161
	CTR-males	45.53	19.91			
	FLX-males	18.05	4.94	-0.74		.505

Hiding alone	CTR-females	104.34	18.76			
	FLX-females	81.65	11.73		1.03	.322
	CTR-males	30.22	15.11			
	FLX-males	18.43	5.07	0.00		1.00
Hiding socially	CTR-females	37.09	9.42			
	FLX-females	33.69	12.65	-0.11		.959
	CTR-males	14.11	5.83			
	FLX-males	10.49	4.13	-0.53		.645
Following	CTR-females	16.46	10.73			
	FLX-females	13.04	5.03	38.50		.505
	CTR-males	225.45	47.93			
	FLX-males	189.71	26.42		0.65	.524
Allogrooming	CTR-females	2.60	1.13			
	FLX-females	1.32	0.86	-0.95		.382
	CTR-males	14.52	12.05			
	FLX-males	3.48	2.04	0.12		1.00
Sniffing anogenitally	CTR-females	131.42	8.31			
	FLX-females	163.09	12.31		-2.13	.051
	CTR-males	391.86	44.56			
	FLX-males	342.34	58.26		0.68	.511
Sniffing nose-to-nose	CTR-females	95.47	21.93			
	FLX-females	90.55	15.42	0.21		.878
	CTR-males	36.72	8.95			
	FLX-males	26.41	5.62	-0.53		.645
All Sniffing	CTR-females	359.95	22.20			
	FLX-females	443.76	39.20	-1.06		.384 (.307)
	CTR-males	621.96	54.81			
	FLX-males	544.49	68.76		0.88	.655 (.393)
Sniffing body	CTR-females	169.06	21.64			
	FLX-females	190.11	21.71		-0.69	.503
	CTR-males	193.38	12.49			
	FLX-males	175.74	18.63		0.79	.445
Fighting	CTR-females	58.83	12.02			
	FLX-females	58.35	9.77		0.03	.976
	CTR-males	25.54	6.00			
	FLX-males	45.19	11.92		-1.47	.163
Nose-off	CTR-females	0.00	0.00			
	FLX-females	0.05	0.05	1.00		.721
	CTR-males	15.06	12.69			
	FLX-males	6.00	2.42	0.86		.442
Self-grooming	CTR-females	94.22	12.49			
	FLX-females	70.47	6.77		1.67	.195 (.117)
	CTR-males	65.17	25.25			
	FLX-males	64.55	16.50	0.53		.806 (.645)
Freezing	CTR-females	7.73	3.59			
	FLX-females	11.48	4.56	1.01		.328
	CTR-males	5.99	2.28			
	FLX-males	12.14	3.37		-1.51	.153
Rearing supported	CTR-females	316.19	25.25			
	FLX-females	343.17	27.60		-0.72	.483
	CTR-males	145.94	17.53			
	FLX-males	129.84	18.44		0.63	.537
Rearing unsupported	CTR-females	11.44	3.14			
	FLX-females	14.28	2.59		- 0.70	.497

	CTR-males	15.04	5.62			
	FLX-males	19.07	3.55	0.58		.574

Note. The data represent the time spent (s) performing all behaviors. Data are analyzed with either Mann-Whitney U test (Z) or t-test (t). P-values before Benjamini-Hochbeg correction are shown in parentheses. Significant differences are highlighted in bold.

Table B1

Results From Mann-Whitney U-Test or t-Test on Frequency of Behaviors

Behavior	Rats	Mean	SEM	Z	t	P
Socially active behaviors	CTR-females	335.38	32.04			
	FLX-females	382.63	32.48		-1.04	.318
	CTR-males	299.00	36.51			
	FLX-males	294.00	31.64		0.10	.919
General activity	CTR-females	1001.75	93.17			
	FLX-females	1209.63	66.07		-1.82	.090
	CTR-males	516.00	44.24			
	FLX-males	590.25	13.64		-1.60	.131
Non-socially passive behaviors	CTR-females	51.63	8.14			
	FLX-females	55.00	6.17		-0.12	.903
	CTR-males	44.63	4.97			
	FLX-males	35.50	5.94		0.62	.546
Socially passive behaviors	CTR-females	12.00	2.67			
	FLX-females	13.00	2.38		-0.28	.784
	CTR-males	11.50	3.08			
	FLX-males	6.75	0.90	1.48		.176
Conflict behaviors	CTR-females	32.13	3.90			
	FLX-females	34.75	5.20		-0.40	.693 (.693)
	CTR-males	11.38	2.68			
	FLX-males	17.13	3.98	1.05		.786 (.328)
Walking/running	**CTR-females**	**290.63**	**28.31**			
	FLX-females	**447.00**	**22.95**		**-4.29**	**.005 (.001)**
	CTR-males	310.75	29.29			
	FLX-males	366.38	18.47		-1.61	.130
Chasing	CTR-females	0.13	0.13			
	FLX-females	0.00	0.00	-1.00		.721
	CTR-males	1.88	1.36			
	FLX-males	2.13	1.33	0.41		.721
Non-social exploration	CTR-females	711.13	71.09			
	FLX-females	762.63	65.02		-0.54	.693 (.601)
	CTR-males	205.25	21.68			
	FLX-males	223.88	13.72		-0.73	.786 (.480)
Digging	CTR-females	27.63	5.23			
	FLX-females	35.75	4.76		-1.15	.270
	CTR-males	4.75	2.06			
	FLX-males	7.13	2.81		-0.68	.506
Resting/immobile alone	CTR-females	2.50	0.95			
	FLX-females	6.13	2.18		-1.53	.149
	CTR-males	28.38	4.23			
	FLX-males	23.75	5.08		0.70	.496

Resting/immobile socially	CTR-females	0.50	0.27			
	FLX-females	2.00	0.80	1.77		.105
	CTR-males	8.38	2.91			
	FLX-males	4.13	1.01	-1.23		.234
Hiding alone	CTR-females	36.63	5.88			
	FLX-females	33.88	4.13		0.38	.708
	CTR-males	4.75	1.54			
	FLX-males	5.00	1.41	0.21		.878
Hiding socially	CTR-females	11.50	2.78			
	FLX-females	11.00	2.41		0.136	.894
	CTR-males	3.13	1.47			
	FLX-males	2.63	1.03	-0.27		.798
Following	CTR-females	5.63	2.10			
	FLX-females	7.00	2.49	0.95		.382
	CTR-males	100.75	22.68			
	FLX-males	81.13	11.30	-0.53		.645
Allogrooming	CTR-females	0.75	0.25			
	FLX-females	0.38	0.18	-1.12		.328
	CTR-males	2.50	1.96			
	FLX-males	1.38	0.75	0.29		.789
Sniffing anogenitally	CTR-females	85.63	7.04			
	FLX-females	104.38	7.01		-1.89	.080
	CTR-males	133.00	22.83			
	FLX-males	116.25	17.69		0.58	.571
Sniffing nose-to-nose	CTR-females	90.13	9.39			
	FLX-females	89.00	6.79		0.10	.924
	CTR-males	37.38	5.98			
	FLX-males	32.13	5.23		0.66	.519
All Sniffing	CTR-females	334.63	32.12			
	FLX-females	382.25	32.40		-1.04	.693 (.314)
	CTR-males	296.50	35.12			
	FLX-males	292.63	31.49		0.08	.936 (.936)
Sniffing body	CTR-females	158.88	24.63			
	FLX-females	188.88	22.77		-0.89	.386
	CTR-males	126.13	9.87			
	FLX-males	144.25	14.73		-1.02	.324
Fighting	CTR-females	32.13	3.90			
	FLX-females	34.63	5.24		-0.38	.708
	CTR-males	9.63	1.87			
	FLX-males	14.63	3.35		-1.30	.213
Nose-off	CTR-females	0.00	0.00			
	FLX-females	0.13	0.13	1.00		.721
	CTR-males	1.75	0.90			
	FLX-males	2.50	0.82	0.87		.442
Self-grooming	CTR-females	24.63	3.75			
	FLX-females	22.00	2.51		0.58	.693 (.570)
	CTR-males	6.50	1.51			
	FLX-males	7.88	1.73		-0.60	.860 (.559)
Freezing	CTR-females	4.63	2.38			
	FLX-females	5.50	1.55	0.96		.382
	CTR-males	2.13	0.69			
	FLX-males	2.63	0.63	0.86		.442
Rearing supported	CTR-females	170.50	17.15			
	FLX-females	181.25	14.63		-0.48	.641

	CTR-males	69.50	9.13		
	FLX-males	64.63	7.69	0.41	.689
Rearing unsupported	CTR-females	9.63	1.60		
	FLX-females	13.63	2.84	-1.23	.240
	CTR-males	9.38	3.36		
	FLX-males	11.38	2.44	-0.48	.638

Note. The data represent the number of instances performing all behaviors. Data are analyzed with either Mann-Whitney U test (Z) or t-test (t). P-values before Benjamini-Hochbeg correction are shown in parentheses. Significant differences are highlighted in bold.

Appendix C

Results From Repeated Measures ANOVA

Table C1

Results of Repeated Measures ANOVA on Duration of Behaviors

Behavior	Rats	Df (error)	Time effect		Time x treatment	
			F	*p*	*F*	*p*
Socially active behaviors	FLX/CTR-females	5 (70)	**4.89**	**< .001**	0.26	.932
	FLX/CTR-males	5 (70)	0.66	.656	0.51	.765
General activity	FLX/CTR-females	2.81 (39.35)	2.06	.124	0.69	.556
	FLX/CTR-males	5 (70)	**7.05**	**< .001**	0.85	.520
Non-socially passive behaviors	FLX/CTR-females	2.23 (31.14)	**3.52**	**.037**	0.26	.795
	FLX/CTR-males	2.33 (32.62)	**3.65**	**.031**	0.58	.592
Socially passive behaviors	FLX/CTR-females	1.20 (16.87)	3.24	.084	0.17	.792
	FLX/CTR-males	1.89 (26.42)	3.16	.061	1.05	.361
Conflict behaviors	FLX/CTR-females	5 (70)	0.34	.889	1.49	.204
	FLX/CTR-males	1.99 (27.88)	2.14	.137	0.98	.388
Walking/running	FLX/CTR-females	5 (70)	**3.02**	**.016**	0.63	.679
	FLX/CTR-males	2.64 (36.92)	1.84	.163	0.69	.547
Chasing	FLX/CTR-females	1 (14)	1.00	.334	1.00	.334
	FLX/CTR-males	1.86 (25.98)	1.48	.246	2.88	.078
Non-social exploration	FLX/CTR-females	5 (70)	**2.49**	**.039**	0.84	.529
	FLX/CTR-males	2.88 (40.20)	**3.99**	**.015**	0.47	.697
Digging	FLX/CTR-females	5 (70)	**4.44**	**< .001**	0.67	.650
	FLX/CTR-males	1.97 (27.51)	2.55	.098	0.30	.739
Resting/immobile alone	FLX/CTR-females	1.48 (20.71)	2.48	.120	0.27	.701
	FLX/CTR-males	2.58 (36.05)	**3.15**	**.043**	0.71	.534
Resting/immobile socially	FLX/CTR-females	1.06 (14.88)	2.91	.107	1.76	.206
	FLX/CTR-males	1.64 (22.89)	3.17	.070	1.68	.211
Hiding alone	FLX/CTR-females	2.04 (28.52)	2.17	.131	0.29	.755
	FLX/CTR-males	1.46 (20.38)	0.70	.466	0.60	.513
Hiding socially	FLX/CTR-females	1.30 (18.14)	2.33	.139	0.33	.628
	FLX/CTR-males	2.13 (29.75)	1.35	.276	0.97	.396
Following	FLX/CTR-females	1.25 (17.49)	2.52	.125	0.38	.592
	FLX/CTR-males	5 (70)	0.26	.935	1.45	.217
Allogrooming	FLX/CTR-females	1.71 (24.00)	1.63	.218	0.44	.616
	FLX/CTR-males	1.51 (21.13)	0.86	.410	0.79	.436
Sniffing anogenitally	FLX/CTR-females	2.94 (41.22)	**3.15**	**.036**	0.14	.935
	FLX/CTR-males	5 (70)	1.53	.220	0.80	.556
Sniffing nose-to-nose	FLX/CTR-females	1.84 (25.82)	3.13	.064	0.88	.418
	FLX/CTR-males	1.97 (27.51)	**4.64**	**.019**	0.38	.682
All sniffing	FLX/CTR-females	5 (70)	**4.92**	**< .001**	0.28	.852
	FLX/CTR-males	5 (70)	0.71	.557	0.58	.638
Sniffing body	FLX/CTR-females	5 (70)	**2.57**	**.034**	0.26	.934
	FLX/CTR-males	5 (70)	1.86	.113	0.17	.973
Fighting	FLX/CTR-females	5 (70)	0.33	.891	1.50	.202
	FLX/CTR-males	2.15 (30.12)	3.08	.058	0.59	.575
Nose-off	FLX/CTR-females	1 (14)	1.00	.334	1.00	.334
	FLX/CTR-males	1.03 (14.40)	1.59	.228	0.83	.382

Self-grooming	FLX/CTR-females	2.26 (31.66)	**4.04**	**.023**	0.50	.633
	FLX/CTR-males	2.35 (32.92)	2.91	.061	1.48	.241
Freezing	FLX/CTR-females	1.14 (15.90)	**4.65**	**.043**	0.10	.790
	FLX/CTR-males	1.84 (25.76)	**3.95**	**.035**	1.89	.173
Rearing supported	FLX/CTR-females	5 (70)	**2.72**	**.027**	1.32	.265
	FLX/CTR-males	5 (70)	**5.31**	**< .001**	0.25	.940
Rearing unsupported	FLX/CTR-females	3.06 (42.81)	1.69	.183	0.08	.972
	FLX/CTR-males	1.88 (26.25)	**4.32**	**.026**	1.68	.208

Note. Summary of the repeated measures ANOVA on time spent (s) on all behaviors over the course of the observational period (data divided into six timebins). Significant effects are highlighted in bold.

Table C2

Results of Repeated Measures ANOVA on Frequency of Behaviors

Behavior	Rats	Df (error)	Time effect		Time x treatment	
			F	*p*	*F*	*p*
Socially active behaviors	FLX/CTR-females	5 (70)	**3.01**	**.016**	0.63	.675
	FLX/CTR-males	5 (70)	1.60	.194	0.99	.416
General activity	FLX/CTR-females	5 (70)	2.08	.078	0.52	.764
	FLX/CTR-males	5 (70)	1.56	.210	1.21	.318
Non-socially passive behaviors	FLX/CTR-females	2.64 (36.99)	1.12	.351	0.18	.889
	FLX/CTR-males	2.56 (35.90)	**3.24**	**.040**	0.51	.649
Socially passive behaviors	FLX/CTR-females	1.72 (24.12)	**4.51**	**.026**	0.41	.636
	FLX/CTR-males	2.04 (28.58)	2.91	.070	1.01	.378
Conflict behaviors	FLX/CTR-females	5 (70)	1.12	.361	0.63	.680
	FLX/CTR-males	1.92	**4.26**	**.026**	0.77	.468
Walking/running	FLX/CTR-females	5 (70)	1.99	.091	0.88	.498
	FLX/CTR-males	2.85 (39.88)	0.69	.554	0.82	.483
Chasing	FLX/CTR-females	1 (14)	1.00	.334	1.00	.334
	FLX/CTR-males	1.72 (24.13)	1.38	.269	2.74	.091
Non-social exploration	FLX/CTR-females	5 (70)	1.68	.150	0.23	.948
	FLX/CTR-males	5 (70)	**5.48**	**< .001**	1.76	.158
Digging	FLX/CTR-females	5 (70)	**5.01**	**< .001**	0.45	.810
	FLX/CTR-males	2.38 (33.33)	1.31	.287	0.96	.405
Resting/immobile alone	FLX/CTR-females	2.99 (41.80)	2.27	.094	1.29	.290
	FLX/CTR-males	2.61 (36.56)	**3.16**	**.042**	0.36	.759
Resting/immobile socially	FLX/CTR-females	1.43 (19.99)	**4.14**	**.043**	1.15	.318
	FLX/CTR-males	1.58 (22.15)	3.11	.074	1.28	.290
Hiding alone	FLX/CTR-females	2.59 (36.27)	0.47	.677	0.33	.779
	FLX/CTR-males	5 (70)	0.43	.828	0.81	.549
Hiding socially	FLX/CTR-females	2.04 (28.53)	3.09	.060	0.48	.627
	FLX/CTR-males	2.41 (33.78)	0.70	.531	0.73	.511
Following	FLX/CTR-females	2.08 (29.16)	1.94	.161	0.24	.795
	FLX/CTR-males	5 (70)	0.16	.988	0.74	.554
Allogrooming	FLX/CTR-females	2.69 (37.60)	1.04	.380	1.04	.380
	FLX/CTR-males	2.50 (31.49)	0.79	.477	0.63	.557
Sniffing anogenitally	FLX/CTR-females	5 (70)	**3.49**	**.024**	0.07	.975
	FLX/CTR-males	5 (70)	1.27	.298	0.84	.527

Sniffing nose-to-nose	FLX/CTR-females	5 (70)	**10.00**	**< .001**	1.44	.240
	FLX/CTR-males	1.92 (26.84)	**6.42**	**.006**	0.16	.845
All sniffing	FLX/CTR-females	5 (70)	**3.01**	**.041**	0.61	.689
	FLX/CTR-males	5 (70)	1.67	.153	0.99	.432
Sniffing body	FLX/CTR-females	5 (70)	**2.58**	**.034**	0.65	.666
	FLX/CTR-males	5 (70)	1.29	.280	1.37	.246
Fighting	FLX/CTR-females	5 (70)	1.06	.388	0.66	.654
	FLX/CTR-males	2.00 (28.06)	**3.57**	**.041**	0.85	.438
Nose-off	FLX/CTR-females	1 (14)	1.00	.334	1.00	.344
	FLX/CTR-males	1.45 (20.31)	**4.57**	**.033**	0.24	.714
Self-grooming	FLX/CTR-females	2.93 (40.99)	**3.08**	**.039**	0.53	.660
	FLX/CTR-males	1.91 (26.80)	2.33	.199	0.86	.432
Freezing	FLX/CTR-females	1.45 (20.36)	**5.01**	**.025**	0.26	.700
	FLX/CTR-males	2.38 (33.34)	**3.90**	**.024**	1.21	.316
Rearing supported	FLX/CTR-females	5 (70)	2.01	.120	1.35	.270
	FLX/CTR-males	5 (70)	**4.02**	**.003**	0.19	.964
Rearing unsupported	FLX/CTR-females	2.68 (37.54)	1.09	.361	0.23	.857
	FLX/CTR-males	1.95 (27.34)	**4.13**	**.028**	1.26	.299

Note. Summary of the repeated measures ANOVA on the number of instances performing the various behaviors over the course of the observational period (data divided into six timebins). Significant effects are highlighted in bold.

Appendix D

Descriptive Statistics For All Timebins

Figure D1

Means and Standard Error for Duration of Behavior Within Timebins

Behavior		10	20	30	40	50	60
Socially active behaviors	CTR-females	80.5 ± 10.9	58.9 ± 6.1	61.4 ± 8.5	75.2 ± 9.5	69.2 ± 8.1	53.4 ± 5.8
	FLX-females	89.0 ± 9.3	67.9 ± 9.0	65.9 ± 5.4	81.4 ± 9.5	86.4 ± 9.0	54.4 ± 10.5
	CTR-males	97.7 ± 13.6	104.3 ± 23.6	120.6 ± 16.8	109.5 ± 17.2	110.3 ± 13.0	94.4 ± 16.9
	FLX-males	79.7 ± 12.9	100.5 ± 25.3	91.2 ± 13.8	77.3 ± 10.9	106.0 ± 14.2	93.6 ± 13.0
General activity	CTR-females	412.7 ± 11.0	400.2 ± 7.4	391.5 ± 11.6	391.4 ± 12.4	361.9 ± 12.7	388.3 ± 19.3
	FLX-females	391.5 ± 8.4	372.7 ± 8.6	394.9 ± 11.8	389.3 ± 5.7	368.3 ± 27.1	390.0 ± 8.0
	CTR-males	362.1 ± 28.6	339.4 ± 27.3	298.1 ± 18.0	267.7 ± 13.9	287.4 ± 28.9	256.4 ± 19.4
	FLX-males	383.1 ± 19.6	336.6 ± 24.0	325 ± 21.6	327.5 ± 9.5	323.9 ± 15.3	308.3 ± 14.3
Non-socially passive behaviors	CTR-females	16.4 ± 4.7	12.3 ± 3.3	14.3 ± 4.0	16.4 ± 4.4	36.8 ± 13.2	30.0 ± 10.0
	FLX-females	12.7 ± 2.8	11.9 ± 2.1	15.6 ± 2.3	14.2 ± 5.0	25.3 ± 13.0	26.8 ± 4.5
	CTR-males	21.1 ± 5.9	19.1 ± 5.1	27.9 ± 6.3	46.0 ± 14.1	39.4 ± 18.8	60.8 ± 16.8
	FLX-males	18.5 ± 6.8	13.4 ± 3.1	17.8 ± 3.9	36.0 ± 11.6	14.5 ± 4.1	34.6 ± 10.2
Socially passive behaviors	CTR-females	2.5 ± 1.4	6.3 ± 3.2	3.2 ± 1.1	4.2 ± 1.1	16.9 ± 8.5	5.1 ± 2.7
	FLX-females	2.8 ± 1.5	3.9 ± 1.9	8.1 ± 3.4	4.9 ± 1.7	18.1 ± 10.6	3.8 ± 1.6
	CTR-males	4.1 ± 2.2	3.3 ± 2.7	2.6 ± 1.6	15.4 ± 5.3	14.9 ± 8.2	19.3 ± 8.7
	FLX-males	2.6 ± 1.3	2.5 ± 1.1	3.9 ± 1.3	7.3 ± 3.2	4.6 ± 1.4	7.7 ± 1.8
Conflict behaviors	CTR-females	8.2 ± 2.6	5.9 ± 1.7	11.4 ± 4.1	14.1 ± 4.8	11.2 ± 3.3	8.1 ± 1.8
	FLX-females	8.4 ± 2.0	12.6 ± 3.1	10.7 ± 3.0	7.9 ± 1.5	8.1 ± 2.5	10.9 ± 3.7
	CTR-males	1.5 ± 0.9	1.9 ± 0.9	3.8 ± 1.7	9.3 ± 3.7	4.6 ± 2.6	19.5 ± 13.4
	FLX-males	2.0 ± 1.1	4.2 ± 3.1	10.9 ± 4.0	16.5 ± 7.2	9.5 ± 2.2	8.7 ± 4.6
Walking/running	CTR-females	74.1 ± 8.1	79.7 ± 14.7	63.2 ± 6.2	68.1 ± 7.7	65.4 ± 9.7	54.4 ± 7.9
	FLX-females	111.6 ± 10.9	116.3 ± 14.7	115.2 ± 18.0	95.5 ± 10.5	102.2 ± 24.7	75.7 ± 13.1
	CTR-males	157.3 ± 17.3	155.5 ± 13.1	131.8 ± 11.9	143.4 ± 6.4	129.0 ± 14.6	122.3 ± 6.7
	FLX-males	176.7 ± 18.7	175.8 ± 14.0	162.8 ± 13.1	186.4 ± 10.2	185.5 ± 16.6	154.8 ± 13.7
Chasing	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.03	0.0 ± 0.0	0.0 ± 0.0
	FLX-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	CTR-males	2.8 ± 1.8	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	FLX-males	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.3	3.6 ± 2.4	0.0 ± 0.0	1.0 ± 0.7
Non-social exploration	CTR-females	338.7 ± 16.2	320.5 ± 8.8	328.3 ± 10.5	323.3 ± 18.0	296.4 ± 11.8	333.9 ± 23.1
	FLX-females	279.9 ± 10.7	256.3 ± 11.7	279.6 ±14.6	293.8 ± 13.1	266.1 ± 26.1	314.3 ± 15.4
	CTR-males	204.8 ± 36.1	183.9 ± 29.2	166.3 ± 16.9	124.3 ± 11.0	158.5 ± 22.5	134.0 ± 19.9
	FLX-males	206.4 ± 31.6	160.9 ± 13.5	162.5 ± 13.9	141.0 ± 16.9	138.3 ± 14.6	153.5 ± 16.2
Digging	CTR-females	5.5 ± 3.2	31.0 ± 11.6	26.0 ± 4.8	17.8 ± 5.6	14.7 ± 4.1	16.9 ± 4.8
	FLX-females	12.6 ± 3.5	28.0 ± 7.6	32.2 ± 7.5	14.6 ± 2.2	15.7 ± 2.2	29.9 ± 8.1
	CTR-males	0.8 ± 0.8	2.4 ± 1.7	16.7 ± 8.3	8.5 ± 7.0	3.0 ± 1.8	2.9 ± 1.9
	FLX-males	4.7 ± 2.8	6.4 ± 3.0	13.3 ± 8.7	5.3 ± 4.0	2.9 ± 1.8	3.6 ± 2.2
Resting/immobile alone	CTR-females	1.1 ± 0.7	0.0 ± 0.0	3.6 ± 3.3	0.5 ± 0.3	7.9 ± 6.4	8.8 ± 8.3
	FLX-females	1.0 ± 1.0	0.3 ± 0.3	3.1 ± 1.9	4.0 ± 2.6	4.6 ± 3.8	11.8 ± 4.4
	CTR-males	17.5 ± 6.4	16.4 ± 5.6	24.7 ± 6.6	34.9 ± 8.6	33.5 ± 17.5	57.1 ± 17.5
	FLX-males	15.3 ± 6.6	11.8 ± 3.1	14.6 ± 4.2	32.2 ± 11.7	13.4 ± 4.1	29.1 ± 10.1
Resting/immobile socially	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.8 ± 0.4	0.0 ± 0.0
	FLX-females	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	1.2 ± 0.7	6.4 ± 4.0	0.2 ± 0.2
	CTR-males	2.0 ± 0.9	1.4 ± 0.8	0.4 ± 0.3	9.5 ± 4.9	14.1 ± 8.4	18.2 ± 9.0
	FLX-males	2.4 ± 1.4	0.7 ± 0.5	2.4 ± 1.2	4.7 ± 2.9	2.8 ± 1.2	

							5.0 ± 1.5
Hiding alone	CTR-females	15.3 ± 4.6	12.3 ± 3.3	10.7 ± 3.1	16.0 ± 4.5	28.8 ± 9.6	21.3 ± 5.7
	FLX-females	11.7 ± 2.2	11.6 ± 2.2	12.5 ± 2.5	10.2 ± 3.9	20.7 ± 9.4	15.0 ± 4.1
	CTR-males	3.5 ± 2.1	2.8 ± 1.1	3.2 ± 2.2	11.1 ± 10.1	5.9 ± 2.1	3.7 ± 2.2
	FLX-males	3.2 ± 1.5	1.6 ± 0.9	3.2 ± 1.7	3.8 ± 1.4	1.1 ± 0.5	5.5 ± 3.2
Hiding socially	CTR-females	2.5 ± 1.4	6.3 ± 3.2	3.2 ± 1.1	3.9 ± 1.2	16.1 ± 8.4	5.1 ± 2.7
	FLX-females	2.8 ± 1.5	3.9 ± 1.9	7.9 ± 3.4	3.7 ± 1.9	11.8 ± 8.3	3.6 ± 1.6
	CTR-males	2.1 ± 2.1	2.0 ± 2.0	2.3 ± 1.7	5.9 ± 2.5	0.8 ± 0.5	1.1 ± 0.8
	FLX-males	1.9 ± 1.9	1.8 ± 1.2	1.5 ± 0.9	2.5 ± 1.5	1.8 ± 1.4	2.8 ± 1.2
Following	CTR-females	8.2 ± 6.1	1.7 ± 1.3	1.7 ± 1.0	1.9 ± 1.4	1.3 ± 1.0	1.6 ± 1.3
	FLX-females	4.6 ± 1.9	2.3 ± 1.4	1.6 ± 0.6	1.8 ± 1.2	1.5 ± 1.0	1.3 ± 0.9
	CTR-males	33.3 ± 9.0	30.8 ± 7.8	36.5 ± 10.3	48.8 ± 12.7	39.7 ± 8.5	36.5 ± 10.5
	FLX-males	30.7 ± 5.6	36.5 ± 6.7	34.4 ± 5.3	26.1 ± 6.0	32.2 ± 6.5	30.6 ± 5.9
Allogrooming	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.8	1.4 ± 1.1	0.2 ± 0.2
	FLX-females	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.9	0.2 ± 0.2
	CTR-males	1.5 ± 1.0	3.7 ± 3.0	1.0 ± 1.0	2.3 ± 1.6	1.8 ± 1.5	4.4 ± 4.4
	FLX-males	1.2 ± 0.8	1.2 ± 1.2	0.0 ± 0.0	0.2 ± 0.2	0.7 ± 0.4	0.2 ± 0.2
Sniffing anogenitallt	CTR-females	35.9 ± 9.0	20.7 ± 2.3	20.1 ± 5.8	19.0 ± 5.4	20.9 ± 3.6	14.8 ± 2.2
	FLX-females	39.7 ± 5.4	27.7 ± 5.8	23.5 ± 5.1	29.6 ± 7.7	25.2 ± 5.1	17.7 ± 3.7
	CTR-males	52.9 ± 12.1	64.1 ± 19.7	85.9 ± 14.7	68.7 ± 13.2	68.1 ± 9.6	52.5 ± 11.6
	FLX-males	38.2 ± 10.7	70.8 ± 21.1	62.3 ± 14.4	43.8 ± 10.4	66.3 ± 13.2	61.1 ± 8.9
Sniffing nose-to-nose	CTR-females	13.5 ± 5.0	10.5 ± 3.8	16.3 ± 8.2	21.4 ± 4.2	18.1 ± 1.7	15.7 ± 2.6
	FLX-females	10.4 ± 2.3	9.4 ± 0.8	14.5 ± 2.2	18.3 ± 3.1	27.5 ± 10.8	10.3 ± 2.0
	CTR-males	3.5 ± 0.7	3.6 ± 1.1	4.6 ± 1.5	7.5 ± 2.2	8.8 ± 3.7	8.8 ± 1.8
	FLX-males	3.6 ± 1.2	2.2 ± 1.1	3.5 ± 1.0	5.0 ± 0.9	6.4 ± 2.3	5.7 ± 1.3
All sniffing	CTR-females	80.5 ± 10.9	58.9 ± 6.1	61.4 ± 8.5	74.3 ± 9.5	67.8 ± 7.4	53.1 ± 5.8
	FLX-females	89.0 ± 9.5	67.6 ± 8.8	65.9 ± 5.4	81.4 ± 9.5	85.6 ± 9.2	54.3 ± 5.8
	CTR-males	96.2 ± 14.4	100.6 ± 23.4	119.6 ± 16.5	107.2 ± 16.7	108.6 ± 12.4	90.0 ± 14.3
	FLX-males	78.5 ± 13.1	99.3 ± 24.4	91.2 ± 13.8	77.1 ± 11.0	105.3 ± 14.4	93.4 ± 13.0
Sniffing body	CTR-females	31.2 ± 5.3	27.7 ± 5.1	25.0 ± 3.9	33.8 ± 6.4	28.8 ± 5.0	22.6 ± 3.0
	FLX-females	39.1 ± 6.0	30.5 ± 3.7	27.9 ± 3.9	33.5 ± 3.5	32.9 ± 5.4	26.2 ± 6.3
	CTR-males	40.0 ± 3.0	33.0 ± 4.8	29.2 ± 6.5	31.0 ± 4.6	31.7 ± 6.7	28.7 ± 4.1
	FLX-males	36.8 ± 4.9	26.3 ± 4.1	25.3 ± 2.7	28.2 ± 4.8	32.6 ± 3.0	26.7 ± 4.9
Fighting	CTR-females	8.2 ± 2.6	5.9 ± 1.7	11.4 ± 4.1	14.1 ± 4.8	11.2 ± 3.3	8.1 ± 1.8
	FLX-females	8.4 ± 2.0	12.6 ± 3.1	10.7 ± 3.0	7.8 ± 1.5	8.1 ± 2.5	10.9 ± 3.7
	CTR-males	1.5 ± 0.9	1.9 ± 0.9	3.8 ± 1.7	8.3 ± 3.3	4.2 ± 2.5	5.8 ± 2.1
	FLX-males	2.0 ± 1.1	4.2 ± 3.1	10.9 ± 4.0	15.1 ± 6.7	7.2 ± 2.0	5.8 ± 2.8
Nose-off	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	FLX-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	CTR-males	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.6	0.4 ± 0.2	13.7 ± 12.4
	FLX-males	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 01	1.3 ± 0.6	2.3 ± 1.3	2.9 ± 1.9
Self-grooming	CTR-females	8.5 ± 3.2	5.3 ± 1.6	20.2 ± 5.8	12.1 ± 3.4	19.0 ± 7.6	29.1 ± 10.9
	FLX-females	3.4 ± 1.1	6.9 ± 1.3	8.8 ± 2.4	15.0 ± 5.1	11.2 ± 3.1	25.3 ± 7.4
	CTR-males	1.0 ± 0.6	7.3 ± 4.4	3.4 ± 2.3	9.6 ± 5.0	21.9 ± 11.7	22.3 ± 9.5
	FLX-males	1.2 ± 0.8	2.6 ± 1.1	16.7 ± 5.8	19.0 ± 9.5	10.3 ± 5.8	15.1 ± 6.0
Freezing	CTR-females	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	5.3 ± 3.2	1.6 ± 0.8	0.4 ±0.2
	FLX-females	0.0 ± 0.0	0.1 ± 0.1	0.6 ± 0.4	6.9 ± 4.1	2.9 ± 1.1	0.9 ± 0.4
	CTR-males	0.9 ± 0.6	0.0 ± 0.0	0.2 ± 0.2	3.6 ± 1.9	0.5 ± 0.3	0.9 ± 0.5
	FLX-males	0.2 ± 0.2	0.0 ± 0.0	0.6 ± 0.6	4.0 ± 2.6	0.3 ± 0.2	7.1 ± 3.0
Rearing supported	CTR-females	41.7 ± 5.9	62.5 ± 6.2	52.3 ± 8.7	47.7 ± 9.3	53.8 ± 7.6	58.2 ± 8.8
	FLX-females	60.3 ± 8.7	80.8 ± 9.2	51.3 ± 5.2	51.4 ± 10.4	50.1 ± 5.9	49.1 ± 6.3
	CTR-males	20.9 ± 3.0	37.7 ± 8.1	26.8 ± 4.2	24.8 ± 6.6	16.7 ± 4.9	19.5 ± 4.3
	FLX-males	20.9 ± 3.4	36.4 ± 7.5	26.1 ± 6.1	16.9 ± 5.4	16.0 ± 4.7	

							13.5 ± 2.1
Rearing unsupported	CTR-females	0.9 ± 0.2	1.9 ± 1.2	1.8 ± 0.7	1.5 ± 0.5	3.6 ± 1.2	1.9 ± 0.6
	FLX-females	0.7 ± 0.3	2.5 ± 1.4	2.1 ± 1.9	2.1 ± 0.9	3.9 ± 1.2	3.0 ± 1.2
	CTR-males	0.5 ± 0.4	0.5 ± 0.3	3.9 ± 1.8	4.6 ± 1.9	1.7 ± 0.8	3.8 ± 1.8
	FLX-males	0.3 ± 0.3	1.7 ± 1.3	4.4 ± 1.8	1.6 ± 0.7	1.6 ± 0.7	9.7 ± 4.0

Note. The data represent the time spent (s) performing all behaviors measured within the six timebins. Data are shown in mean ± standard error of the mean.

Figure D2

Means and Standard Error for Frequency of Behavior Within Timebins

Behavior		10	20	30	40	50	60
Socially active behaviors	CTR-females	59.0 ± 10.7	55.1 ± 7.0	49.4 ± 4.9	66.9 ± 9.3	56.5 ± 5.3	48.5 ± 3.6
	FLX-females	69.5 ± 6.6	68.1 ± 8.7	62.8 ± 5.1	67.4 ± 5.8	66.5 ± 9.2	48.3 ± 8.2
	CTR-males	47.9 ± 8.7	44.6 ± 7.0	54.0 ± 8.5	51.1 ± 8.1	50.5 ± 5.1	51.0 ± 9.2
	FLX-males	42.4 ± 6.7	45.1 ± 8.7	46.9 ± 3.9	47.0 ± 5.3	61.4 ± 6.6	51.3 ± 4.9
General activity	CTR-females	159.0 ± 24.1	175.5 ± 22.1	171.4 ± 21.9	177.4 ± 18.5	162.6 ± 17.6	155.9 ± 9.3
	FLX-females	204.8 ± 17.8	224.3 ± 14.9	213.8 ± 9.3	207.4 ± 15.4	189.8 ± 16.9	169.3 ± 22.8
	CTR-males	92.5 ± 6.4	98.6 ± 9.6	88.8 ± 10.0	83.9 ± 8.8	76.8 ± 8.3	75.5 ± 9.4
	FLX-males	97.3 ± 6.9	99.3 ± 4.9	100.5 ± 5.3	98.3 ± 5.2	97.6 ± 6.1	97.5 ± 5.3
Non-socially passive behaviors	CTR-females	5.5 ± 0.3	5.6 ± 1.0	5.6 ± 1.6	6.6 ± 1.5	7.8 ± 2.0	8.0 ± 2.3
	FLX-females	5.5 ± 1.0	5.5 ± 0.9	7.4 ± 1.3	6.4 ± 1.5	7.3 ± 1.6	8.0 ± 1.7
	CTR-males	3.9 ± 0.8	3.9 ± 0.6	4.1 ± 1.1	6.3 ± 1.6	7.0 ± 2.6	8.0 ± 1.5
	FLX-males	4.3 ± 1.6	2.9 ± 0.6	4.0 ± 0.7	6.5 ± 1.7	3.9 ± 1.0	7.3 ± 2.0
Socially passive behaviors	CTR-females	1.0 ± 0.3	2.0 ± 0.6	1.4 ± 0.5	1.9 ± 0.4	3.8 ± 1.2	2.0 ± 0.9
	FLX-females	1.1 ± 0.4	1.8 ± 0.7	2.5 ± 0.8	1.5 ± 0.4	4.5 ± 1.4	1.6 ± 0.7
	CTR-males	1.3 ± 0.8	0.9 ± 0.6	0.5 ± 0.2	2.4 ± 0.7	3.6 ± 1.6	2.9 ± 1.1
	FLX-males	0.5 ± 1.2	0.6 ± 0.3	1.0 ± 0.3	1.6 ± 0.7	1.3 ± 0.3	1.8 ± 0.3
Conflict behaviors	CTR-females	3.8 ± 0.9	5.0 ± 1.2	4.9 ± 1.2	7.8 ± 1.0	5.8 ± 1.9	5.0 ± 0.8
	FLX-females	5.4 ± 1.5	6.5 ± 1.6	6.5 ± 1.3	6.5 ± 1.1	4.9 ± 1.1	5.0 ± 1.8
	CTR-males	0.8 ± 0.4	1.4 ± 0.5	1.6 ± 0.5	3.1 ± 1.1	2.6 ± 0.9	1.9 ± 0.8
	FLX-males	0.9 ± 0.5	1.1 ± 0.5	2.8 ± 0.5	6.3 ± 2.7	3.9 ± 1.1	2.4 ± 0.9
Walking/running	CTR-females	44.4 ± 7.6	52.6 ± 8.5	51.1 ± 8.1	50.4 ± 6.5	46.1 ± 7.2	46.0 ± 4.5
	FLX-females	75.4 ± 3.0	88.5 ± 8.9	81.6 ± 4.7	73.3 ± 5.3	71.6 ± 9.9	56.6 ± 10.7
	CTR-males	52.5 ± 5.4	57.8 ± 6.9	51.8 ± 7.3	53.3 ± 6.2	46.6 ± 5.1	48.9 ± 6.9
	FLX-males	54.6 ± 6.4	61.6 ± 4.3	61.5 ± 4.0	65.4 ± 5.1	64.4 ± 5.8	59.1 ± 4.5
Chasing	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	FLX-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	CTR-males	1.8 ± 1.3	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	FLX-males	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	1.4 ± 0.9	0.0 ± 0.0	0.5 ± 0.4
Non-social exploration	CTR-females	114.6 ± 17.0	122.9 ± 15.8	120.3 ± 14.4	127.0 ± 13.9	116.5 ± 11.2	109.9 ± 8.9
	FLX-females	129.4 ± 15.0	135.8 ± 12.3	132.1 ± 12.0	134.1 ± 12.9	118.1 ± 13.9	112.6 ± 13.7
	CTR-males	40.0 ± 3.8	40.9 ± 5.8	37.0 ± 3.8	30.6 ± 4.1	30.1 ± 4.9	26.6 ± 3.4
	FLX-males	42.6 ± 3.3	37.6 ± 2.8	39.0 ± 2.7	32.9 ± 2.9	33.3 ± 3.3	38.4 ± 4.2
Digging	CTR-females	2.9 ± 1.4	6.4 ± 1.9	6.1 ± 1.5	3.5 ± 1.1	3.4 ± 0.7	5.4 ± 1.1
	FLX-females	5.0 ± 1.7	8.6 ± 1.7	8.8 ± 2.2	2.5 ± 0.7	3.4 ± 0.5	6.5 ± 1.3
	CTR-males	0.3 ± 0.3	0.6 ± 0.4	0.9 ± 0.4	1.4 ± 1.0	0.9 ± 0.5	0.8 ± 0.5
	FLX-males	0.9 ± 0.5	1.6 ± 0.7	2.5 ± 1.4	1.4 ± 0.8	0.5 ± 0.3	0.4 ± 0.2
Resting/immobile alone	CTR-females	0.4 ± 0.3	0.0 ± 0.0	0.4 ± 0.3	0.5 ± 0.3	0.9 ± 0.4	0.4 ± 0.3
	FLX-females	0.3 ± 0.3	0.1 ± 0.1	1.0 ± 0.6	1.5 ± 0.9	1.1 ± 0.5	2.1 ± 0.9

	CTR-males	3.1 ± 0.9	3.4 ± 0.6	3.6 ± 1.1	5.3 ± 1.1	5.6 ± 2.2	7.4 ± 1.6
	FLX-males	3.4 ± 1.5	2.3 ± 0.6	3.3 ± 0.7	5.5 ± 1.6	3.4 ± 1.0	6.0 ± 1.9
Resting/immobile socially	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	0.0 ± 0.0
	FLX-females	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.5 ± 0.3	1.3 ± 0.7	0.1 ± 0.1
	CTR-males	0.5 ± 0.2	0.5 ± 0.3	0.3 ± 0.2	1.4 ± 0.7	3.3 ± 1.7	2.5 ± 1.2
	FLX-males	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.2	1.0 ± 0.6	0.9 ± 0.4	1.1 ± 0.2
Hiding alone	CTR-females	5.1 ± 0.5	5.6 ± 1.0	5.3 ± 1.6	6.1 ± 1.6	6.9 ± 1.9	7.6 ± 2.2
	FLX-females	5.3 ± 1.0	5.4 ± 0.9	6.4 ± 1.3	4.9 ± 1.4	6.1 ± 1.2	5.9 ± 1.7
	CTR-males	0.8 ± 0.4	0.5 ± 0.2	0.5 ± 0.3	1.0 ± 0.7	1.4 ± 0.5	0.6 ± 0.3
	FLX-males	0.9 ± 0.4	0.6 ± 0.3	0.8 ± 0.4	1.0 ± 0.3	0.5 ± 0.2	1.3 ± 0.7
Hiding socially	CTR-females	1.0 ± 0.3	2.0 ± 0.6	1.4 ± 0.5	1.8 ± 0.5	3.4 ± 1.1	2.0 ± 0.9
	FLX-females	1.1 ± 0.4	1.8 ± 0.7	2.4 ± 0.8	1.0 ± 0.4	3.3 ± 1.2	1.5 ± 0.7
	CTR-males	0.8 ± 0.8	0.4 ± 0.4	0.3 ± 0.2	1.0 ± 0.3	0.4 ± 0.3	0.4 ± 0.3
	FLX-males	0.1 ± 0.1	0.4 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.4 ± 0.3	0.6 ± 0.3
Following	CTR-females	2.0 ± 0.8	0.8 ± 0.5	0.6 ± 0.3	0.9 ± 0.4	0.6 ± 0.4	0.8 ± 0.5
	FLX-females	1.9 ± 0.7	1.3 ± 0.6	1.1 ± 0.6	0.8 ± 0.5	1.1 ± 0.7	0.9 ± 0.5
	CTR-males	16.6 ± 4.9	14.0 ± 3.2	17.9 ± 4.9	20.1 ± 5.3	16.9 ± 4.0	15.3 ± 5.1
	FLX-males	12.9 ± 2.9	15.1 ± 3.1	14.0 ± 1.8	12.0 ± 2.5	13.4 ± 2.4	13.6 ± 2.8
Allogrooming	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3	0.3 ± 0.2	0.1 ± 0.1
	FLX-females	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
	CTR-males	0.4 ± 0.3	0.6 ± 0.5	0.3 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.8 ± 0.8
	FLX-males	0.4 ± 0.3	0.4 ± 0.4	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	0.1 ± 0.1
Sniffing anogenitallt	CTR-females	20.8 ± 5.1	15.6 ± 1.6	13.1 ± 3.1	13.6 ± 2.6	12.6 ± 1.6	9.9 ± 1.0
	FLX-females	22.9 ± 2.5	20.3 ± 3.4	17.1 ± 2.8	16.6 ± 2.8	15.8 ± 3.0	11.8 ± 2.3
	CTR-males	19.5 ± 5.2	20.1 ± 4.4	28.0 ± 5.8	23.3 ± 5.5	21.9 ± 3.9	20.4 ± 5.8
	FLX-males	14.4 ± 3.4	21.5 ± 5.4	20.8 ± 3.4	16.3 ± 3.1	22.9 ± 3.4	20.5 ± 2.8
Sniffing nose-to-nose	CTR-females	11.4 ± 1.9	11.5 ± 2.1	12.6 ± 1.8	21.1 ± 2.7	16.6 ± 1.1	16.9 ± 2.2
	FLX-females	11.5 ± 1.1	12.1 ± 1.2	15.4 ± 1.4	19.8 ± 3.3	18.0 ± 1.7	12.1 ± 1.8
	CTR-males	4.4 ± 0.8	4.8 ± 1.3	4.5 ± 1.1	6.6 ± 1.3	8.5 ± 2.5	8.6 ± 0.9
	FLX-males	3.9 ± 1.0	3.3 ± 1.2	4.1 ± 1.0	6.4 ± 1.0	7.5 ± 1.9	7.0 ± 1.2
All sniffing	CTR-females	59.0 ± 10.7	55.1 ± 7.0	49.4 ± 4.9	66.5 ± 9.3	56.3 ± 5.2	48.4 ± 3.6
	FLX-females	69.5 ± 6.6	68.0 ± 8.6	62.8 ± 5.1	67.4 ± 5.8	66.4 ± 9.2	48.1 ± 8.2
	CTR-males	47.5 ± 8.6	44.0 ± 6.7	53.8 ± 8.4	50.9 ± 8.0	50.3 ± 5.1	50.3 ± 8.7
	FLX-males	42.0 ± 6.6	44.8 ± 8.5	46.9 ± 3.9	46.9 ± 5.3	61.0 ± 6.6	51.1 ± 4.9
Sniffing body	CTR-females	26.9 ± 5.8	28.0 ± 5.6	23.6 ± 4.1	31.8 ± 6.4	27.0 ± 3.8	21.6 ± 2.3
	FLX-females	35.1 ± 5.3	35.6 ± 4.9	30.3 ± 4.1	31.0 ± 3.2	32.6 ± 5.7	24.3 ± 5.1
	CTR-males	23.6 ± 3.1	19.1 ± 1.6	21.3 ± 3.5	21.0 ± 3.2	19.9 ± 2.3	21.3 ± 2.8
	FLX-males	23.8 ± 4.4	20.0 ± 3.1	22.0 ± 1.9	24.3 ± 3.5	30.6 ± 3.3	23.6 ± 2.6
Fighting	CTR-females	3.8 ± 0.9	5.0 ± 1.2	4.9 ± 1.2	7.8 ± 1.0	5.8 ± 1.9	5.0 ± 0.8
	FLX-females	5.4 ± 1.5	6.5 ± 1.6	6.5 ± 1.3	6.4 ± 1.1	4.9 ± 1.1	5.0 ± 1.8
	CTR-males	0.8 ± 0.4	1.4 ± 0.5	1.6 ± 0.5	2.4 ± 0.7	2.3 ± 0.8	1.3 ± 0.5
	FLX-males	0.9 ± 0.5	1.1 ± 0.5	2.6 ± 0.5	5.1 ± 2.2	3.1 ± 0.9	1.8 ± 0.7
Nose-off	CTR-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	FLX-females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	CTR-males	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.5	0.4 ± 0.2	0.6 ± 0.4
	FLX-males	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	1.1 ± 0.5	0.8 ± 0.3	0.6 ± 0.4
Self-grooming	CTR-females	2.5 ± 0.5	3.0 ± 1.1	4.5 ± 1.0	4.1 ± 1.0	5.0 ± 1.4	5.5 ± 1.2
	FLX-females	2.3 ± 0.7	3.3 ± 0.8	3.6 ± 0.5	3.5 ± 1.0	3.0 ± 0.9	6.4 ± 1.6
	CTR-males	0.4 ± 0.3	0.9 ± 0.3	0.6 ± 0.4	1.4 ± 0.7	1.3 ± 0.4	2.0 ± 0.6
	FLX-males	0.3 ± 0.2	0.6 ± 0.3	1.9 ± 0.6	2.4 ± 1.3	1.3 ± 0.5	1.5 ± 0.5
Freezing	CTR-females	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.3	2.6 ± 1.6	1.0 ± 0.6	0.5 ± 0.3
	FLX-females	0.0 ± 0.0	0.1 ± 0.1	0.6 ± 0.4	2.1 ± 1.0	1.6 ± 0.5	1.0 ± 0.3
	CTR-males	0.3 ± 0.2	0.0 ± 0.0	0.3 ± 0.3	1.0 ± 0.5	0.3 ± 0.2	0.4 ± 0.2

	FLX-males	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.3	0.8 ± 0.4	0.3 ± 0.2	1.3 ± 0.4
Rearing supported	CTR-females	25.6 ± 5.3	32.0 ± 5.0	27.1 ± 4.4	25.9 ± 4.6	29.8 ± 3.3	30.1 ± 3.7
	FLX-females	34.9 ± 4.7	40.1 ± 3.7	27.1 ± 2.1	27.4 ± 4.8	24.8 ± 4.1	27.0 ± 3.5
	CTR-males	10.3 ± 2.1	16.5 ± 2.5	12.6 ± 2.4	11.9 ± 3.0	8.9 ± 1.9	9.3 ± 2.6
	FLX-males	10.9 ± 2.7	16.3 ± 3.2	12.4 ± 2.4	8.8 ± 2.1	8.0 ± 2.0	8.0 ± 1.3
Rearing unsupported	CTR-females	1.3 ± 0.3	1.4 ± 0.6	1.6 ± 0.4	1.1 ± 0.3	2.8 ± 0.8	1.5 ± 0.6
	FLX-females	0.9 ± 0.3	2.3 ± 1.0	2.6 ± 2.1	2.5 ± 1.2	3.4 ± 0.9	2.0 ± 0.7
	CTR-males	0.5 ± 0.3	0.4 ± 0.2	2.4 ± 1.1	2.8 ± 1.0	1.0 ± 0.4	2.4 ± 1.1
	FLX-males	0.1 ± 0.1	1.1 ± 0.7	3.3 ± 1.2	1.0 ± 0.4	1.0 ± 0.4	4.9 ± 2.2

Note. The data represent the number of instances performing all behaviors measured within the six timebins. Data are shown in mean ± standard error of the mean.