Third-party prosocial behavior in adult female rats is impaired after perinatal fluoxetine exposure

Indrek Heinla[1], Roy Heijkoop[1], Danielle J. Houwing[1,2], Jocelien D.A. Olivier[2], Eelke M.S. Snoeren[1,3*]

[1] Department of Psychology, UiT The Arctic University of Norway, Norway

[2] Groningen Institute for Evolutionary Life Sciences, University of Groningen, The Netherlands

[3] Regional Health Authority of North Norway

*** Correspondence:**

Eelke.snoeren@uit.no

Eelke Snoeren, Department of Psychology, UiT the Arctic University of Norway, Postboks 6050 Langnes, 9037 Tromsø, Norway

Keywords: perinatal; fluoxetine; SSRI; seminatural environment; prosocial; consolation

Short title: Fluoxetine exposure impairs prosocial behavior

Abstract

SSRIs are commonly used to treat pregnant women with depression. However, SSRIs can cross the placenta and affect the development of the fetus. The effects of perinatal SSRI exposure, and especially the effects on social behavior, are still incompletely documented. This study first aims to investigate whether rats show prosocial behavior in the form of consolation behavior. Secondly, it aims to investigate whether perinatal SSRI exposure affects this prosocial behavior. At last, we investigate whether the behavior changed after the rats had been exposed to an additional white-noise stressor.

Rat dams received 10 mg/kg/d fluoxetine (FLX) or vehicle (CTR) via oral gavage from gestational day 1 until postnatal day 21. At adulthood, the rat offspring were housed in four cohorts of 4 females and 4 males in a seminatural environment. As prosocial behaviors are more prominent after stressful situations, we investigated the behavioral response of rats immediately after natural aggressive encounters (fights). Additionally, we studied whether a stressful white-noise exposure would alter this response to the aggressive encounters.

Our study indicates that CTR-female rats are able to show third party prosocial behavior in response to witnessing aggressive encounters between conspecifics in a seminatural environment. In addition, we showed that perinatal FLX exposure impairs the display of prosocial behavior in female rats. Moreover, we found no signs of prosocial behavior in CTR- and FLX-males after natural aggressive encounters. After white-noise exposure the effects in third party prosocial behavior of CTR-females ceased to exist. We conclude that female rats are able to show prosocial behavior, most likely in the form of consolation behavior. In addition, the negative effects of perinatal fluoxetine exposure on prosocial behavior could provide additional evidence that SSRI treatment during pregnancy could contribute to the risk for social impairments in the offspring.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are the most prevalent treatment for women with depression during pregnancy [1, 2]. Nonetheless, since SSRIs can cross the placenta and appear in breastmilk [3-5], the question rises how this treatment might affect the developing fetus. SSRI exposure during development affects the serotoninergic system in the fetus [5, 6]. While serotonin acts as neurotransmitter at adulthood, it is a neurotrophic factor during early brain development. More specifically, perinatal SSRI exposure can affect the regulation of cell division, differentiation, migration, and synaptogenesis [7, 8]. Whether or not these changes also have an effect later in life remains unclear, but it is assumed that perinatal SSRI exposure affects the subsequent serotonergic function and vulnerability to affective disorders [9].

Several studies have found an association between perinatal SSRI exposure and disturbed sleep patterns, affected social-emotional development, and increased internalizing and externalizing behavior in the offspring [10-12]. Recently, an ongoing debate started about whether children whose mothers were treated with SSRIs during pregnancy have an increased risk to develop autism spectrum disorder (ASD). Some studies show a clear correlation between SSRI treatment and increased odds for ASD in the offspring [13-17], whereas others did not find this link or suggest that this increased risk is caused by the depression itself rather than the SSRIs [18, 19]. The mothers who do take antidepressants during pregnancy most likely suffer from a more severe depression, and untreated depression during pregnancy may also have negative impact on the offspring [18, 20-23]. In fact, when controlled for maternal mood and stress, the link between antenatal SSRI use and the occurrence of ASD in the offspring does not persist [24].

Where human epidemiological studies struggle with the lack of control over variables and possible confounding factors, animal studies can provide a more fundamental insight into underlying mechanisms of illnesses and treatments. By treating healthy mothers with

antidepressants, one can discern the effects of the drug exposure during pregnancy on neurodevelopmental alterations in the offspring.

Although the link with ASD and perinatal SSRI exposure remains controversial, effects of perinatal SSRI exposure on social behavior have been found. For example, some studies showed that both pre- and early postnatal SSRI treatment can decrease social play behavior and communicative skills (ultrasonic vocalizations) in young rodent offspring [25-29], with the exception of one study showing an increase in social play [30]. In terms of social interaction at adulthood, the findings are more controversial. Some studies have indicated that developmental SSRI exposure decreases social interaction in either male offspring [25, 27] or female offspring [31], while others found an increase in sniffing behavior towards conspecifics [32, 33]. When looking at the motivation for social interaction in particular, the majority found that SSRI exposure negatively affects the motivation to start social contact [26, 28, 29, 34]. The inconsistent results make it impossible to draw conclusions about the risk for long-term affected social behavior in the offspring. In addition, the amount of, or motivation for social exploration does not include the role of passive social behavior, which could also lead to social relationships. Recently, we found that both male and female rats that were perinatally exposed to fluoxetine more often spent passive moments in the company of a conspecific (social resting) compared with control rats [31], confirming the risk for affected social behavior resulting from perinatal SSRI exposure.

Interestingly, another important element of social behavior which received less attention in research is *prosocial behavior*. Prosocial behavior is defined as behavior that is intended to benefit another individual, and includes helping and consolation behavior [35]. The difference between helping and consolation behavior is that consolation is an increase in affiliative contact (like grooming and hugging) in response to and directed toward a distressed individual by a

bystander, which produces a calming effect [36]. Helping behavior, on the other hand, also improves the status quo of another individual, but does not require direct contact.

Until now, consolation behavior has been documented in several species (including great apes, dogs, wolves, rooks, elephants, and prairie voles), mainly in the context of naturally occurring aggressive conflicts [36-42]. With regard to helping behavior, several studies have been performed in rats. Rats do liberate conspecifics trapped in restrainers or soaked in water arenas, even when there is no social reward at the end of the test [43-45]. When rats were placed in a food-foraging task, they behave prosocially by choosing the option that provides their cage mate with food as well [46]. However, no studies have yet confirmed that rats show consolation behavior, especially not in a more natural setting.

This study, therefore, first aims to investigate whether rats show prosocial behavior in the form of consolation behavior. The second aim of the study was to investigate whether perinatal SSRI exposure affects this prosocial behavior. At last, we investigate whether the behavior changed after the rats had been exposed to an additional white-noise stressor. We used a seminatural environment in which cohorts of eight rats are group housed for 8 days. The advantage of this environment is that the rats are able to express their full repertoire of natural behaviors. If consolation behavior exists in rats, it is expected to happen after stressful events like natural occurring aggressive encounters between conspecifics. We, therefore, observed the behavior of all rats during the 15 minutes after fights and expected to find an increase in active social behavior directed at conspecifics. In a previous study, we found that phenotypes in perinatally SSRI exposed offspring can alter after experiencing a stressful event [31]. We, therefore, also investigated whether the performance of prosocial behavior is changed after an additional stressor.

2. Materials and methods

This study describes the data from subsequent analyses of video recordings from another study. This means that the rats and thus the procedures were the same as mentioned in [31], but the behavioral observations were chosen and designed specifically for the purpose of the current study. The materials and methods describe the steps that were required for the current study.

2.1 Housing conditions

Male (n=10) and female Wistar rats (n=10), weighing 200-250g on arrival, were obtained from Charles River (Sulzfeld, Germany). They were used as dams and potential fathers of the offspring, and housed in same sex pairs in Makrolon IV cages (60 × 38 × 20 cm) under a 12 : 12 h reversed light/dark cycle (lights on 23.00 h) at 21 ± 1 °C and 55 ± 10% relative humidity. Standard rodent food pellets (standard chow, Special Diets Services, Witham, Essex, UK) and tap water were available ad libitum, and nesting material was present.

On the day of conception, each female was housed with one male in a Makrolon IV cage for 24 hours, after which she returned to her same sex pair for the following two weeks of gestation. On gestational day 14, the females were single-housed in Makrolon IV cages for delivery, in which they stayed until weaning of the pups.

The offspring were housed together with their mother until weaning (postnatal day 21). Thereafter until the start of the experiment, the offspring were housed in groups of two/three same sex littermates in Makrolon IV cages. Ears were punched for individual recognition. The animals were left undisturbed except during cage cleaning.

All experimentation was carried out in agreement with the European Union council directive 2010/63/EU. The protocol was approved by the National Animal Research Authority in Norway.

2.2 Breeding and fluoxetine treatment

Each female was checked daily for sexual receptivity by placing the females together with a male rat for a maximum of 5 minutes. When a lordosis response was observed, the female was considered in estrus and placed with the male for approximately 24 hours (Gestational day 0).

From gestational day 1 (G1) until postnatal day 21 (PND21), a total of 6 weeks, the mothers of the experimental animals were treated daily with a stainless steel feeding needle per oral gavage with either fluoxetine (10 mg/kg) or vehicle (Methylcellulose 1%, (Sigma, St. Louis, MO, USA)) in a volume of 5 ml/kg. Fluoxetine pills were crushed and dissolved in sterile water (2 mg/ml), while 1% methylcellulose was dissolved directly in sterile water. The females were weighed every three days to determine the dose for the following three days. The dose of fluoxetine was based on comparison to human treatment [27, 47]. Near the end of pregnancy, dams were checked twice per day (9 p.m. and 15 p.m.) for delivery. An overview of the whole procedure from the beginning of antidepressant treatment until the end of testing of the offspring is presented in Figure 1.

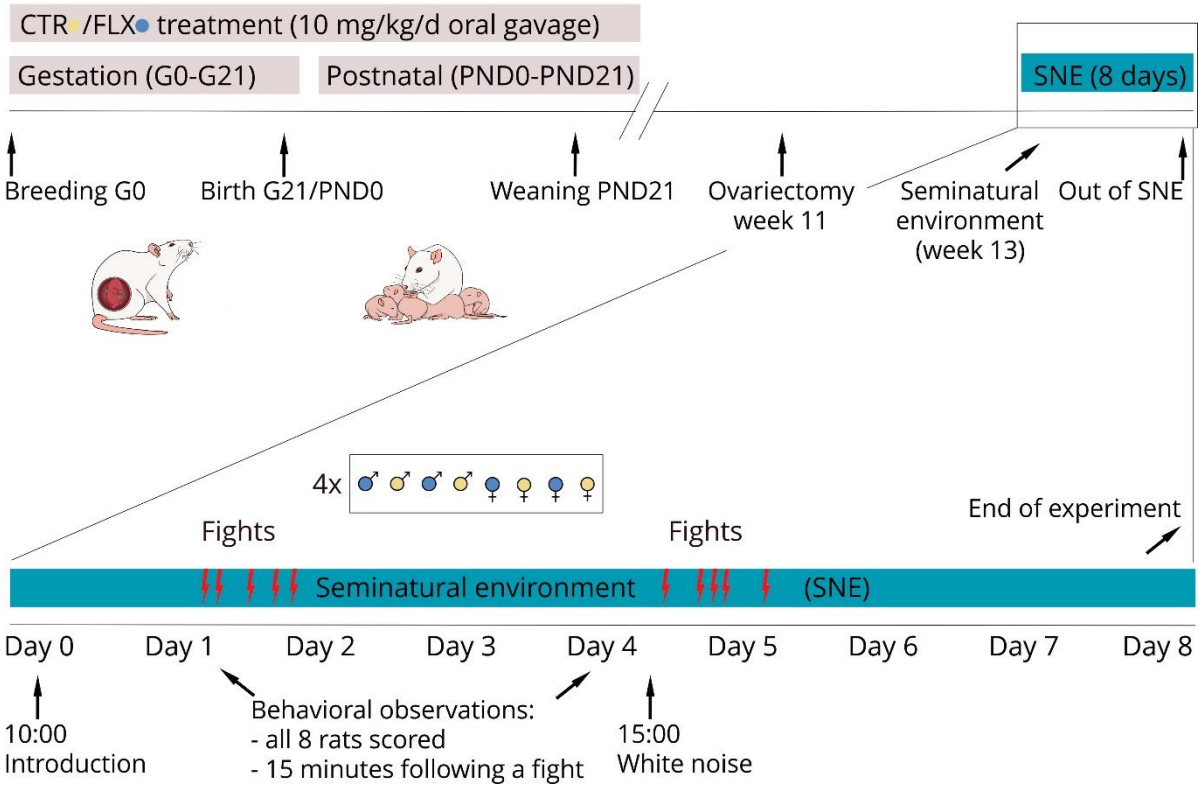

Figure 1. Timeline of the experiment. Pregnant rats were treated via oral gavage with either vehicle (CTR) or 10 mg/kg/d fluoxetine (FLX) from gestational day 0 (G0) until weaning of the pups (PND21). The behavior of the offspring at adulthood was evaluated in the seminatural environment (SNE) after naturally occurring aggressive encounters (fights). An additional stressor, a 10-minute white-noise exposure, was presented in day 4, enabling the comparison between baseline and post-stress behavior.

2.3 Design of the study

As mentioned, the offspring were housed in groups of two/three same sex littermates after weaning and left undisturbed until an age of 13-18 weeks (adulthood). From these offspring, 32 rats distributed in 4 cohorts were used for behavioral evaluation in the seminatural environment. A cohort of rats consisted of two male offspring from control mothers (CTR-males), two males from fluoxetine-treated mothers (FLX-males), two females from control mothers (CTR-females) and two females from fluoxetine-treated mothers (FLX-females). The total of 4 cohorts thus resulted in n=8 per treatment and sex group for data analysis.

Within a cohort, same sex rats came from different litters and were therefore unfamiliar to each other. However, due to a limited amount of litters available, some animals had one sibling from the opposite sex in the same cohort. These littermates had been housed in different home cages since weaning. Details of the litter distribution in the cohorts can be found in the supplemental materials of [31].

For the purpose of the previous study [31], the females had undergone ovariectomy two weeks before entering the seminatural environment. This procedure was irrelevant for the purpose of the current study. It only has the consequence that the females were in diestrus of the menstrual cycle at the moment of behavioral observations in the current study.

2.4 Procedure in the seminatural environment

The day before the subjects were introduced into the seminatural environment, they were shaved on the back and tail-marked under isoflurane anesthesia for individual recognition. (For more details, see [31]) In addition, the offspring were weighed and no differences in body weight were found between CTR and FLX animals.

Each cohort of rats was introduced into the seminatural environment on the first day (Day 0) at 10 a.m., and removed at the end of the experiment on day 8 at 10 a.m. Between cohorts, the seminatural environment was thoroughly cleaned to remove olfactory cues from previous animals.

2.5 Description of the seminatural environment

The seminatural environment (2.4 x 2.1 x 0.75 meters) setup is previously described in [31, 48, 49]. It consists of a burrow system and an open field area, which are connected by four 8 x 8 cm openings. The burrow system consists of an interconnected tunnel maze (7.6 cm wide and

8 cm high) with 4 nest boxes (20 x 20 x 20 cm), and is covered with Plexiglas. The open area is an open area with 0.75 meter high walls, and contained two partitions (40 x 75 cm) to simulate obstacles in nature. A curtain between the burrow and the open field allowed the light intensity for both arenas to be controlled separately. The burrow system remained in total darkness for the duration of the experiment, while a day-night cycle was simulated in the open area with a lamp 2.5 m above the center that provided 180 lux from 22.45h to 10.30h (the equivalent of daylight) and approximately 1 lux from 10.30h to 11.00h (the equivalent of full moonlight). The light gradually increased/decreased during 30 minutes between 1 and 180 lux.

The floors of both the open area and on the burrow system were covered with a 2 cm layer of aspen wood chip bedding (Tapvei, Harjumaa, Estonia). In addition, the nest boxes were provided with 6 squares of nesting material each (nonwoven hemp fibres, 5 x 5 cm, 0.5 cm thick, Datesend, Manchester, UK), and in the open area 3 red polycarbonate shelters (15 x 16.5 x 8.5 cm, Datesend, Manchester, UK) were placed and 12 aspen wooden sticks (2 x 2 x 10 cm, Tapvei, Harjumaa, Estonia) were randomly distributed. Food was provided in one large pile of approximately 2 kg in the open area close to the water supply. Water was available ad libitum in 4 water bottles.

Two video cameras (Basler) were mounted on the ceiling 2 meter above the seminatural environment: one above the open field and an infrared video camera above the burrow system. Videos were recorded using Media recorder 2.5. by direct connection to a computer allowing the data to be stored immediately on an external hard drive. Every 24 hours, the recording was manually stopped and restarted to create recordings with a length of 24h. This was done to assure that if a recording error would occur, data from only one day would be lost.

2.6 White-noise

To investigate the response of the offspring to a stressful event, and compare the behavior before and after, the rats were exposed to 90dB white-noise containing all frequencies at the same time, produced by a white-noise generator (Lafayette instruments, Lafayette, IN). This white-noise was played to the rats via two loudspeakers (Scan-Speak Discovery 10F/8414G10, HiFi Kit Electronic, Stockholm), one of which was placed in the open field and the other in the burrow area. Exposure occurred on day 4 at 15.00h and lasted for 10 minutes.

2.7 Behavioral observations

In order to find potential prosocial behavior after naturally occurring stressful situations, the video recordings were screened for aggressive encounters between the animals. An aggressive encounter was registered when two rats attempted to bite and/or wrestle each other aggressively, usually accompanied by loud high-pitch screeching, resulting in one animal (loser) fleeing the area. Boxing, nose-off and playful wrestling were not considered aggressive encounters.

For each aggressive encounter, the role of each rat during this encounter was determined with 4 possibilities: 1) winner - chasing the conspecific after the encounter; 2) loser - running away, escaping from the winner; 3) witness - was in immediate vicinity of the fight and/or paid attention to the ongoing fight (by facing the fight) or 4) non-witness - was not in immediate vicinity of the fight and did not pay attention to the ongoing fight.

The behavior of each cohort member was observed during 15 minutes immediately after each aggressive encounter. Originally, we planned to include aggressive encounters at two different time points: A) five fights at baseline (the pre-stress condition, starting on day 1 of the experiment) and B) five fights after the white-noise exposure (the post-stress condition, starting immediately after the white-noise exposure on day 4). However, in order to test our hypothesis,

the most important comparison is to investigate the behavior of each rat in instances when they witnessed aggressive encounters versus instances when they did not. Since the fights were not sufficient to expose enough rats to both instances, we continued the search for aggressive encounters until most rats had played both roles at least once. This resulted in an outcome of 5-6 fights per rat at baseline, and 5-7 fights in post-stress. However, some rats never played both roles during the encounters, which resulted in a few missing data points.

The duration and/or the frequency of the behaviors defined in table 1a was registered by an observer, blind for the treatment of the animals, in the Observer XT software (Noldus Information Technology, The Netherlands; version 12.5). In addition, we registered towards who social and conflict behaviors were directed, and in what location (open area, tunnels or nest box) the behaviors took place.

Table 1a. Description of recorded behaviors

Behavior	*Description*
Allogrooming	Subject is grooming another rat
Sniffing others	Subject is sniffing another rat's face or body area
Sniffing anogenitally	Subject is sniffing another rat's anogenital area
Self-grooming alone	Subject grooms itself
Self-grooming near others	Subject grooms itself in close proximity of others
Walking	Subject is moving around in the environment
Walking over/under other	Walking over/under other rat
Resting/immobile	Subject is sleeping or standing still with no or minimal head movement
Resting with others	Subject is huddling/resting in the close vicinity of others
Non-social exploration	Subject is actively sniffing around and/or rearing in the environment
Digging/carrying	Subject digs bedding material or moves around nesting material or food pellets
In opening facing open area	Subject is staying in the opening between open area and the burrow and looking towards open area
In opening facing burrow	Subject is staying in the opening between open area and the burrow, looking towards the burrow
Fighting	Subject is fighting with another rat
Nose-off	Subject is facing another rat and aggressively posturing towards it
Defensive posture	Subject is defensively posturing near other rats
Boxing	Subject is facing another rat and frequently hitting it with front paws
Wrestling	Subject wrestles with another rat
Fleeing	Subject is fleeing from another rat
Chasing	Subject chases other rat after a conflict situation

Drinking	Subject drinks
Witnessing	Was in immediate vicinity of the fight and/or paid attention to the ongoing fight
Non witnessing	Was not in immediate vicinity of the fight and did not pay attention to the ongoing fight

Table 1b. Description of behavioral clusters

Cluster	*Behaviors within the cluster*
Active social behavior	Allogrooming, Sniffing others, Sniffing anogenitally
Passive social behavior	Resting with others
Social context	Allogrooming, Sniffing others, Sniffing anogenitally, Self-grooming near others, Resting with others
General activity	Walking, Walking over/under other, Non-social exploration
General passive	Resting/immobile, Resting with others
Conflict	Fighting, Nose-off, Defensive posture, Kicking, Boxing, Wrestling, Fleeing, Chasing
Self-grooming	Self-grooming alone, Self-grooming near others

2.8 Statistical analysis

As shown in Table 1b, behavioral clusters were created for behaviors that fit in the same behavioral group. First, for each rat, we calculated the frequency and the total time spent on each behavior (and behavioral cluster) after each aggressive encounter. Then, we calculated the average of these frequencies and durations of behaviors of all encounters (5-7) per animal. In addition, we calculated the average of the frequencies and durations of behaviors during all encounters taken from the perspective of the role the rat played during the fights (witness versus non-witness) and used them as separate data points as well. This analysis was performed for both baseline and post-stress aggressive encounters.

A Shapiro–Wilk test showed no homogeneity of variance. All behavioral data were therefore analyzed using the nonparametric Mann–Whitney U test to compare FLX-rats with CTR-rats, and witness versus non-witness conditions. The Wilcoxon test, on the other hand, was used to compare baseline with post-stress conditions. $P<.05$ (two-tailed) was considered statistically significant.

3. Results

3.1 Do rats show prosocial behavior after aggressive encounters?

In order to investigate the first aim of the study on whether or not rats show prosocial behavior, we compared the behavior of CTR-rats between instances when they witnessed aggressive encounters (witness) versus instances when they did not (non-witness). The data at baseline revealed that CTR-females that witnessed the aggressive encounters spent significantly more time on active social behaviors than when they did not witness the fights (Z=-2.43; p=.015; *d*=1.232, Figure 2A*)*. When the different behaviors of this behavioral cluster were analyzed separately, we found that witness CTR-females were allogrooming longer (Z=-2.593; p=.010; *d*=1.616, Figure 2B$_1$*)* and more often (trend: Z=-1.893; p=.058; *d*=1.068, Table S1) than non-witness CTR-females. No differences were found in sniffing others (Figure 2B$_2$) and anogenital sniffing (Figure 2B$_3$). Also in terms of passive social behavior, there was no significant difference between witness and non-witness CTR-females (integrated in Figure 3A). However, witness CTR-females showed an increase in the behavioral cluster "social context", which contains all behaviors in the clusters passive social behavior and active social behavior (plus self-grooming near others), compared to non-witnesses (Z=-2.083; p=.040; *d*=1.349, integrated in Figure 4B*)*.

Interestingly, when the receivers of the prosocial behaviors were analyzed in more detail for CTR-females, it was found that active social behavior, including allogrooming, was performed significantly more often and longer towards the conspecifics which did not participate in the aggressive encounter (but did experience the social tensions in the environment) than towards the losers and winners (Witness: time spent sniffing others versus losers: Z=-2,36; p=.018; *d*=1.75; others versus winners: Z=-2.875; p=.004; *d*=2.67; Non-witness: others versus losers: Z=-2.417; p=.016; *d*=1.96; others versus winners: Z=-2.521; p=.012; *d*=2.49; Figure 2C$_1$).

(We should take into account, though, that there are always 5-6 others and only 1 winner, and 1 or 2 losers.)

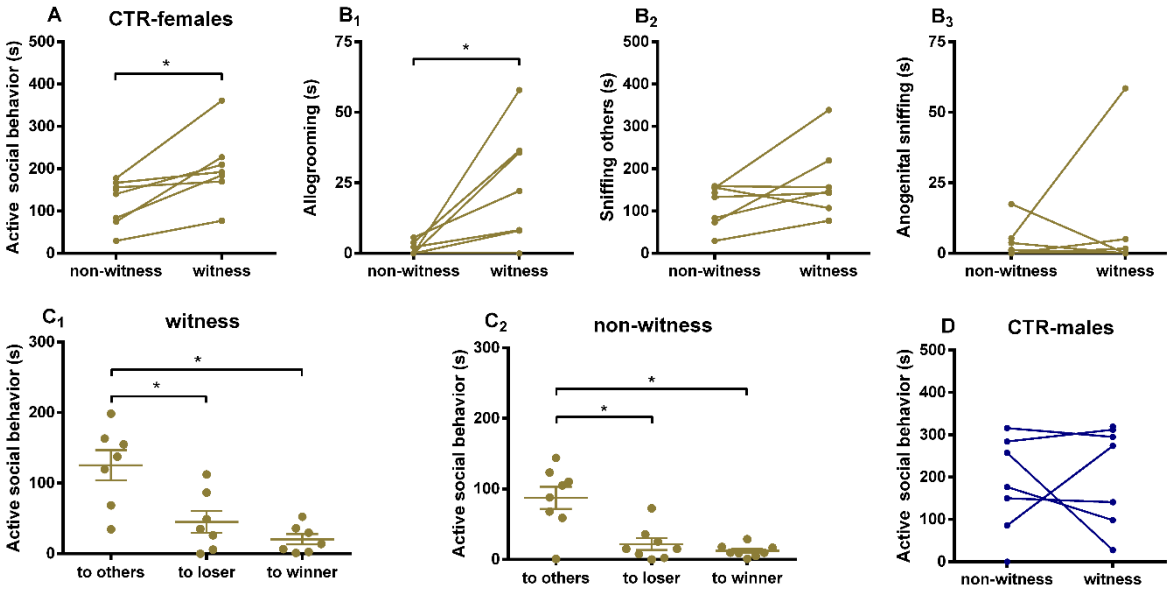

*Figure 2. The data represents the time spent (s) on a behavior by CTR-rats at adulthood in a seminatural environment at baseline (from day 1). The graphs show a comparison between instances when the CTR-rats witnessed aggressive encounters (witness) versus instances when they did not (non-witness). Data are shown as individual data points, with the lines connecting data points from the same rat. * $p<0.05$.*
(A) the behavioral cluster "active social behavior" in CTR-females, (B) the components of active social behavior split up per behavior: allogrooming (B1), sniffing others (B2), and anogenital sniffing (B3) in CTR-females, (C) the active social behavior of witness (C1) and non-witness (C2) CTR-females split up per receiver of the behavior: to others, to losers, and to winners of the aggressive encounters, and (D) active social behavior in CTR-males.

With regard to any other behavior, no differences were found between whether or not CTR-females witnessed the aggressive encounter (integrated in Figure 4C-J). Witnesses spent, for instance, the same amount of time on general activity, passive behavior, and self-grooming as non-witnesses.

Interestingly, CTR-males that witnessed the aggressive encounter did not show different behavior than CTR-males that did not witness the fight (Figure 2D). They spent a similar amount

of time on, for instance, active social behavior, passive social behavior, general activity, passive behavior, and self-grooming (integrated in Figure 4).

3.2 Does perinatal fluoxetine exposure affect prosocial behavior?

The second aim of the study was to investigate the effects of perinatal SSRI-exposure on prosocial behavior. First, we compared the behaviors between FLX-rats between instances when they witnessed aggressive encounters versus instances when they did not at baseline. The data revealed that neither FLX-females nor FLX-males showed any differences in behavior between witness and non-witness instances (Figure 3A/C). Thus, the increase in prosocial behavior found in CTR-females (that was associated with witnessing aggressive encounters) was absent in FLX-females (Figure 3A). While no differences were found in behaviors of non-witness CTR- versus non-witness FLX-females, witness FLX-females spent significantly less time in active social behavior than witness CTR-females after an aggressive encounter ($Z=-2.192$; $p=.030$; $d=1.474$, Figure 3A). When the differences between non-witness and witness instances were calculated per rat and compared between CTR- and FLX-females, the data confirmed that CTR-females showed a significantly larger increase in allogrooming than the FLX-females who actually did not show any increases at all. ($Z=-2.390$; $p=.017$; $d=1.671$, Figure 3B).

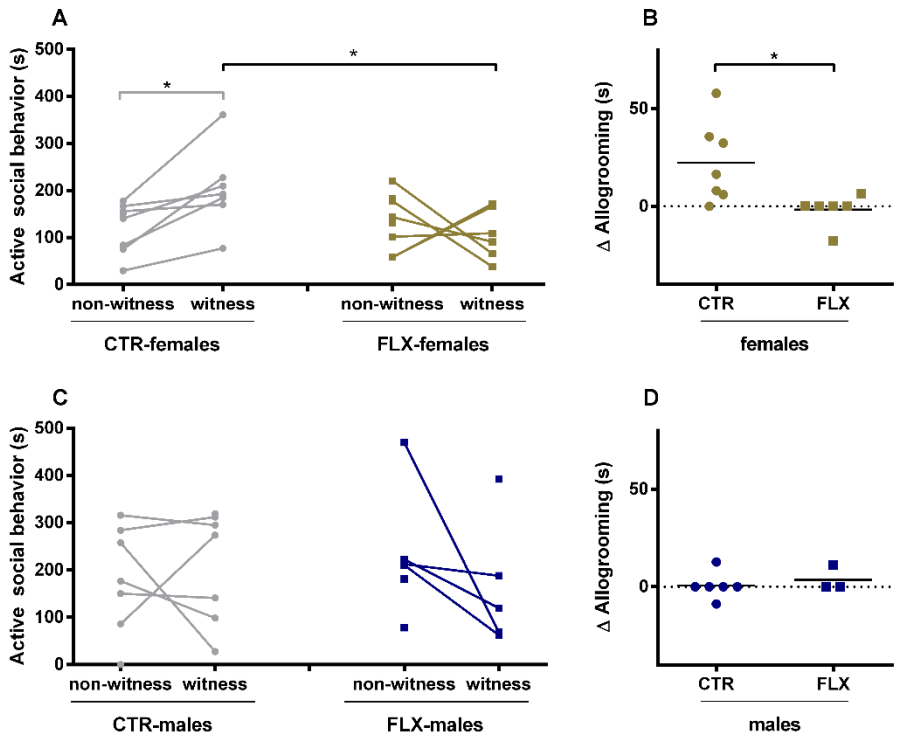

*Figure 3. (A) The data represents the time spent (s) on active social behavior by FLX-females compared to CTR-females (same figure from Figure 1A). The graph shows a comparison between instances when the rats witnessed aggressive encounters (witness) versus instances when they did not (non-witness). (B) The data represents the difference in time spent on allogrooming during instances as witness and as non-witness. The graph shows a comparison between CTR-females and FLX-females. (C/D) Same as figures 2A and B, but now for CTR- and FLX-males. Data are shown as individual data points, with the lines connecting data points from the same rat. * $p<0.05$.*

Again, when the different components of the behavioral cluster "active social behavior" were analyzed individually, FLX-females who witnessed the aggressive encounters did not allogroom more or less compared to the non-witnesses. In addition, witness FLX-females spent significantly less often ($Z=-2.105$; $p=.035$; $d=1.41$, Figure 4A) and shorter time ($Z=-2.314$; $p=.021$; $d=1.67$, Figure 4B) in allogrooming compared to witness CTR-females. For all other behaviors, no differences were found between CTR- and FLX-females, or witness versus non-witness (Figure 4B-J). Likewise, CTR-males and FLX-males showed similar behaviors after aggressive encounters independent of whether they did or did not witness the fight (Table S1).

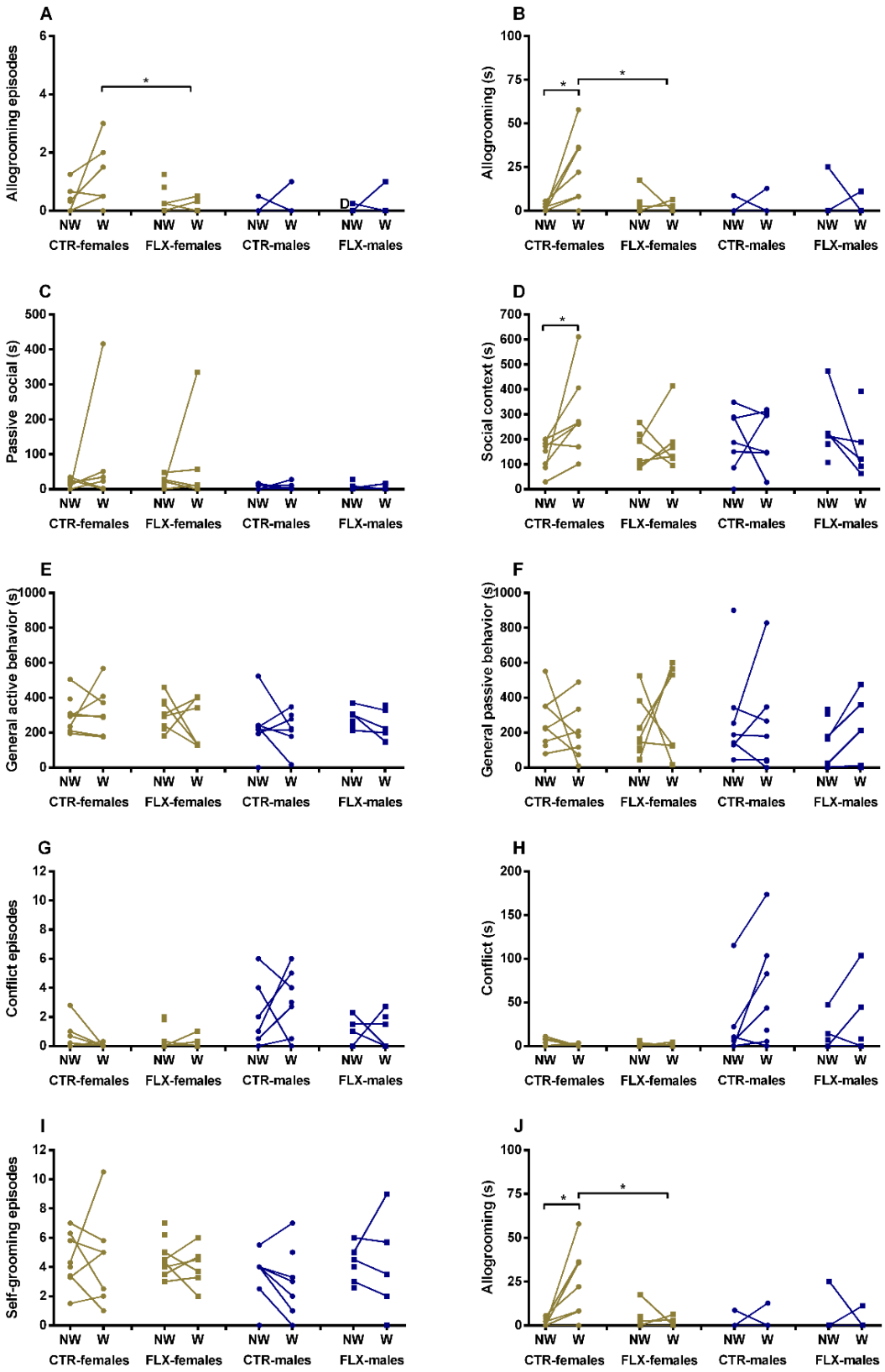

*Figure 4. The data represents either the time spent (s) on a behavior or the number of episodes of the behavior performed by CTR- and FLX-rats (both females and males) at adulthood in a seminatural environment at baseline. The graphs show a comparison between instances when the rats witnessed aggressive encounters (witness) versus instances when they did not (non-witness). Data are shown as individual data points, with the lines connecting data points from the same rat. * $p<0.05$.*
(A) the number of allogrooming episodes, (B) time spent allogrooming, (C) time spent on passive social behavior, (D) time spent in a social context, (E) time spent being generally active, (F) time spent being generally passive, (G) number of conflict episodes, (H) time spent in conflict situations, (I) number of self-groom episodes, and (J) time spent self-grooming.

3.3 What are the effects of an additional stressor on prosocial behavior?

The last aim of the study was to investigate whether an additional stressor (white-noise) can alter the behaviors found in CTR- and FLX-rats. First, we performed the same analysis as mentioned above, but now for the behavior occurring after the aggressive encounters after the 10-minute white-noise exposure on day 4. Surprisingly, following white-noise, we found that CTR-females now did *not* show an increase in active social behavior (or the subcomponent allogrooming) when they witnessed an aggressive encounter, compared to when they did not witness the fight (Figure 5A/B). They also no longer spent more time in a social context (Figure 5C). In fact, witness CTR-females spent significantly less time on passive social behavior compared to non-witness instances ($Z=-2.641$; $p=.008$; $d=1.209$, Figure 5D). In addition, no differences were found in other behaviors like general activity and self-grooming (Figure 5E-H). CTR-males again did not show differences in behaviors between instances in which they witnessed an aggressive encounter or instances in which they did not (Figure 5).

With regard to the FLX-rats, we found no differences in behaviors of FLX-males and FLX-females after the white-noise during instances in which they witness an aggressive encounter versus instances when they did not (Figure 5). Except that FLX-females even allogroomed less during instances of witnessing a fight compared to non-witnessing ($Z=-2.411$; $p=.016$; $d=1.383$, Figure 5B). In addition, witness FLX-females were longer generally active than

non-witness-FLX females (Z=-2.315; p=.021; *d*=1.586, Figure 5E), but this was not different from CTR-female conditions.

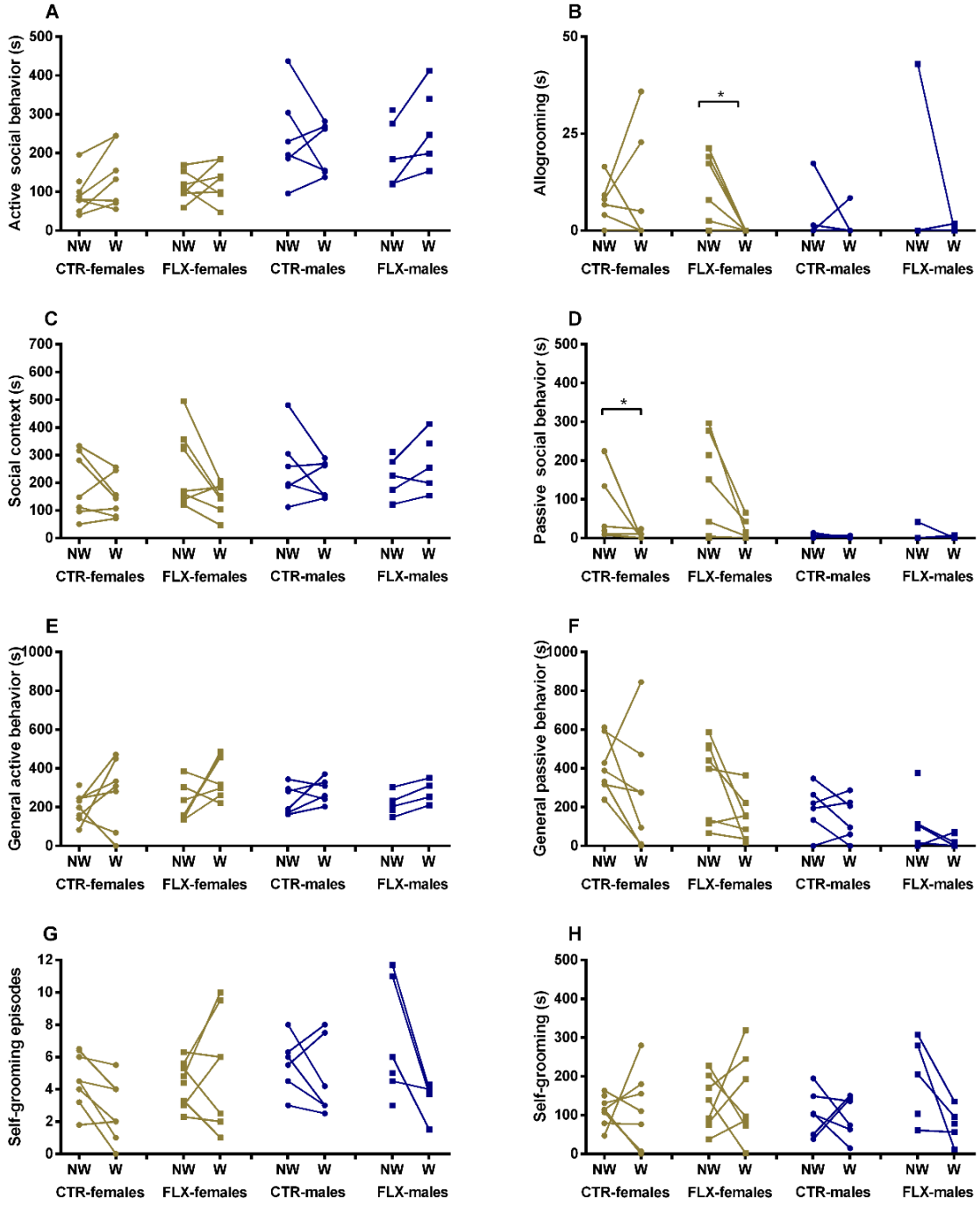

*Figure 5. The data represents either the time spent (s) on a behavior or the number of episodes of the behavior performed by CTR- and FLX-rats (both females and males) at adulthood in a seminatural environment after white-noise exposure (post-stress). The graphs show a comparison between instances when the rats witnessed aggressive encounters (witness) versus instances when they did not (non-witness). Data are shown as individual data points, with the lines connecting data points from the same rat. * $p<0.05$.*
(A) time spent on active social behavior, (B) time spent allogrooming, (C) time spent in social context, (D) time spent on social passive behavior, (E) time spent being generally active, (F) time spent being generally passive, (G) number of self-groom episodes, and (H) time spent self-grooming.

When the behaviors in the post-stress conditions were compared to the baseline measures (for witness and non-witness instances separate), the data revealed that no differences between baseline and post-stress were found on any behavior in CTR-males and CTR-females. With regard to the FLX-rats, also no differences in behavior were found between baseline and post-stress, neither as witness nor as non-witness.

3.4 Does perinatal fluoxetine exposure affect behavior in general after a social stressor?

When the effects of fluoxetine-exposure on behavior after aggressive encounters in general were investigated at baseline, without taking the role of the subjects into account, the data revealed that both FLX-females and FLX-males spent the same amount of time on e.g. active and passive social behaviors, general activity, conflict behavior and self-grooming as CTR-rats (Table S2). Thus, perinatal SSRI exposure does not affect the behavior in response to a naturally occurring aggressive encounter in the seminatural environment. The only differences are found when they actually witnessed the aggressive encounter.

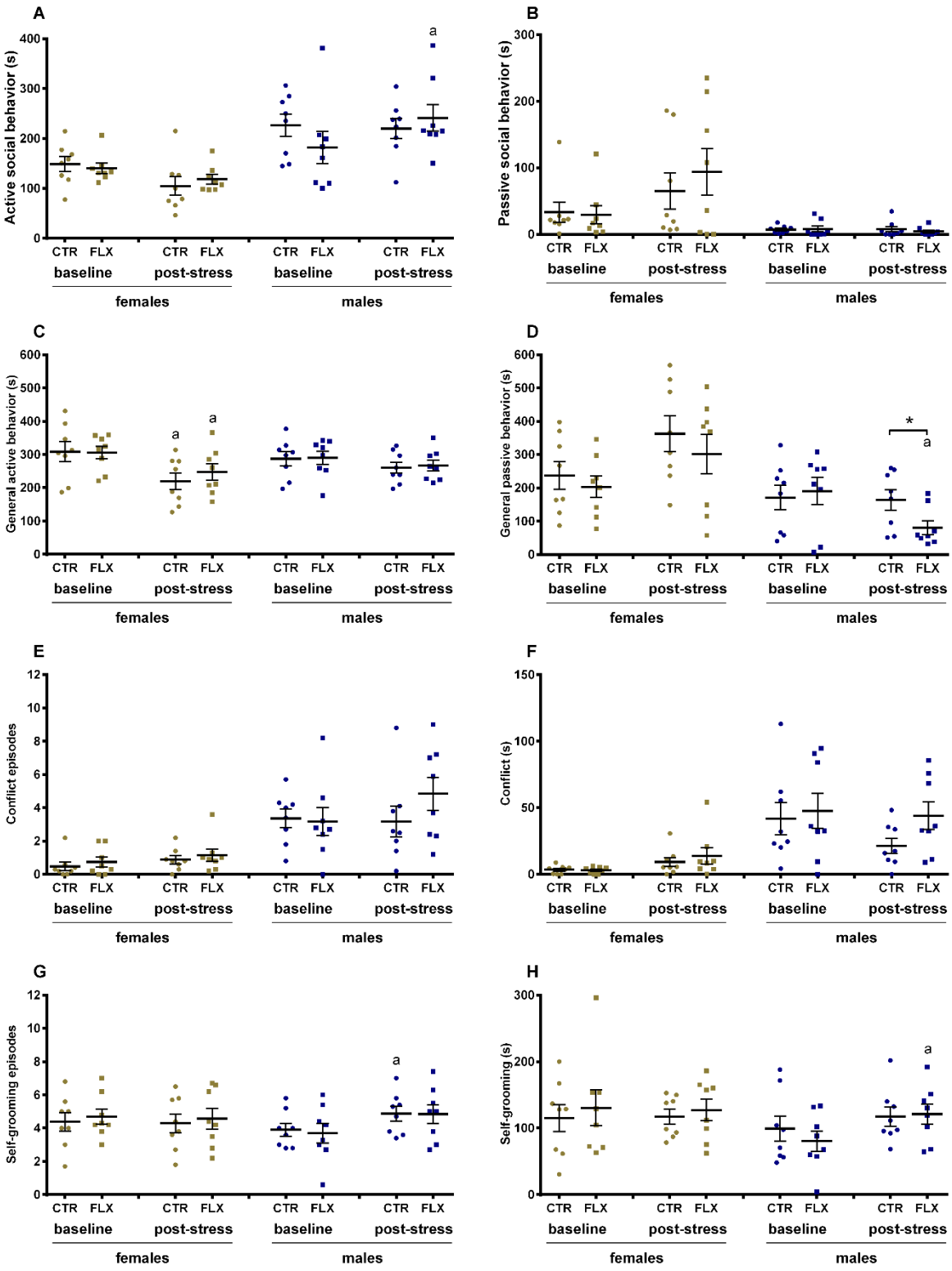

*Figure 6. The data represents either the time spent (s) on a behavior or the number of episodes of the behavior performed by CTR- and FLX-rats (both females and males) at adulthood in a seminatural environment at baseline and after white-noise exposure (post-stress). The graphs show a comparison between CTR- and FLX-rats. Data are shown as individual data points, the line and whiskers represent group mean+/-SEM.. * $p<0.05$ compared to CTR. [a] $p<0.05$ compared to baseline.*

(A) time spent on active social behavior, (B) time spent on passive social behavior, (C) time spent general active, (D) time spent general passive, (E) number of conflict episodes, (F) time spent in conflict situations, (G) number of self-groom episodes, and (H) time spent self-grooming.

Also when the behavior was evaluated after the white-noise exposure, no relevant differences were found between CTR- and FLX-females, or CTR- and FLX-males. FLX-males only seem to spend less time being passive than CTR-males post-stress (Z=-2.143; p=.032; d=1.2, Figure 6).

When the behaviors in the post-stress conditions were compared to the baseline measures, we found that both CTR-and FLX-females were less generally active after the stressor than at baseline (CTR-females: Z=-1.96; p=.05; d=1.22 and FLX-females: Z=-1.96; p=.05; d=1, Figure 6). With regard to the male rats, it was found that an additional stressor caused an increase in active social behavior (Z=-2.521; p=.012; d=.762), social context (Z=-2.521; p=.012; d=.796) and self-grooming (Z=-2.103; p=.035; d=1.009) and a decrease in general passive behavior (Z=-1.963; p=.050; d=1.3) in FLX-males compared to baseline. Regarding CTR-males there was an increase in self-grooming episodes after the stressor (Z=-2.201; p=.028; d=.884). Though, as mentioned above, these increases and decrease did not result in significant differences between CTR- and FLX-males.

4. Discussion

The first aim of the study was to explore if rats are capable of showing prosocial behavior. As mentioned before, prosocial behavior is defined as behavior that is intended to benefit another individual, and includes helping and consolation behavior. In our study, we studied the possibility that rats show consolation behavior, which should be visible as an increase in affiliative contact directed towards presumably distressed conspecifics in response to an aggressive encounter that took place in the close vicinity. Therefore, we compared the behavior of rats that witnessed the aggressive encounters (and were thus able to observe a stressful event resulting in distressed conspecifics, both those involved in the fights and third-party bystanders) with their behavior when they did not witness the fights (and were thus *not* able to notice distressed rats when it happened).

Our results indicate that female rats do indeed show prosocial behavior. CTR-females, but not CTR-males, showed higher levels of social behavior towards conspecifics during instances when they witnessed an aggressive encounter than instances when they did not witness the fight. This suggests that witness CTR-females seek social interaction after a stressful social conflict situation, and thereby most likely either try to seek comfort themselves or offer social relief to their conspecifics who experienced discomfort caused by a social conflict in the environment. Previous studies in other rodent species have suggested that consolation behavior, a form of prosocial behavior, is mostly offered in the form of allogrooming behavior [42, 50]. Besides a general effect in social context and active social behavior, our results also showed an increase in allogrooming behavior after witnessing a fight, suggesting that allogrooming is a form of consolation behavior for female rats. This suggests that the increase in active social behavior, including allogrooming, was likely intended to offer social relief to their conspecifics, and could be considered prosocial behavior.

The second aim of the study was to investigate whether perinatal SSRI exposure can affect the expression of prosocial behavior in rats at an adult age, which could be an indicator for deficits in social behavior. We found that FLX-females, in contrast to CTR-females, did not show any signs of prosocial behavior in our experiment in the seminatural environment. This indicates that perinatal fluoxetine exposure does indeed affect prosocial behavior in female rats. Since CTR-males did not show any signs of prosocial behavior, no conclusions can be drawn whether or not perinatal fluoxetine exposure also affects male prosocial behavior. Though, we can conclude that males and females cope differently with stressors.

The third aim of the study was to see if an additional stressor would change the prosocial behavior. Indeed, the increase in active social behavior in CTR-females that occurred following an aggressive encounter (indicated as prosocial behavior) diminished after a stressful white-noise exposure and therefore the difference between CTR- and FLX-females ceased to exist. Additionally, in response to the combined stressors of white-noise *and* an aggressive encounter, no differences in behavior were found for CTR- and FLX-male rats.

Collectively, these results suggest that the perinatal SSRI exposure has sex- and context-dependent effects on prosocial behavior.

4.1 Prosocial behavior as consolation behavior?

Although our results indicate the existence of prosocial behavior after instances of witnessing aggressive encounters, at least in females, it is unclear whether this prosocial behavior can be called *consolation* behavior. In order to be defined *consolation* behavior, the prosocial behavior should have a stress-lowering effect in the conspecifics [42, 51]. Since we were not able to measure corticosterone levels before and after the aggressive encounters or after the received prosocial behavior, we cannot be absolutely certain that the observed behavior is indeed

consolation behavior. However, the fact that the prosocial behavior is solely seen after instances of *witnessing* an aggressive encounter is in line with studies on consolation behavior by third-party bystanders in different species. In bonobos, for instance, more consolation behavior was shown to be displayed when an animal was closer to a conflict [37]. In addition, the anti-stress effect of allogrooming has previously also been shown reducing heart rates in several species [52-54]. This suggests that the increase in active social behavior, including allogrooming, found in our study was most likely intended to offer social relief to their conspecifics, and could be considered consolation behavior.

In our study, the displayed prosocial behavior was mostly directed at individuals who were themselves not involved in the aggressive encounter (third-party bystanders, or 'others'). CTR-females showed significantly more active social behaviors towards the others than towards the losers and winners. Initially, we expected that consolation behavior would take place towards the losers, as also shown in other the species [37, 38]. However, it is not unthinkable to assume that prosocial behavior is aimed at the other rats who probably also suffered from distress of the social conflict in the environment, resulting in spreading a change in general arousal of rats through the environment. A similar finding was presented for Asian elephants where they offered consolation to other bystanders [40]. Rats who lose the fight might actually prefer to withdraw from social contact after defeat and are therefore not actively pursued. In fact, it has been shown that after a serious social defeat, rats reduce their general activity and refrain from social contact, even with non-aggressive conspecifics [55, 56].

In addition, none of the rats involved in the aggressive encounters in our study suffered visible physical damage nor seemed to change their behavior remarkably. It is, therefore, possible that the spontaneous aggressive encounters in our setting were not severe enough to evoke consolation behavior towards the losers. For sure, it could be the reason why our effects are

rather modest in general, and why we do not observe prosocial behavior in CTR-males. Hypothetically, male rats might need to observe higher levels of distress or different modalities of stress in order to show prosocial behavior to conspecifics. Another explanation could be that a long reciprocal social history is a prerequisite for consolation to occur between animals. Social bonds have been shown to be relevant in other species: bonobos and chimpanzee bystanders were more likely to console relatives or closely bonded partners [37, 57], and unsolicited third-party contact occurred more often in wolves and mice between individuals that shared close relationships [39, 58]. In our study, the rats had been in the same group for less than a week, and only 1 day at baseline. Therefore, another possible reason why the "losers" are not consoled in our study might be that the social ties between the animals are not developed well enough. However, the fact that prosocial behavior was offered to the other rats in the surroundings suggest that this argument is not valid in our set-up, since they are just as unfamiliar to the consolers as the losers. In addition, it was previously shown that rats also rescue strangers from trapped situations [44].

4.2 How does perinatal fluoxetine exposure affect prosocial behavior?

It is evident in our FLX-females that perinatal SSRI exposure affects prosocial behavior. The response to witnessing the aggressive encounter that we discussed above is absent in almost all of the FLX-exposed females. Behavior of FLX-females seems unaltered by instances of witnessing an aggressive encounter, whereas CTR-females react with increased active social behavior, including allogrooming. This indicates possible deficiencies in the prosocial response of FLX-females. The question remains why FLX-females (and FLX-males) lack the "normal" response to witnessing an aggressive encounter? Since our experimental set-up was not designed to answer this question, the answer could potentially be found in previous research. One

hypothesis is that perinatal SSRI exposure alters the responses to stressors via changing control mechanisms involved in the regulation of the negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis. Corticosterone and glucocorticoid play an important role in regulating HPA axis functionality. The hypothesis is supported by a previous study showing that prenatal exposure to fluoxetine can increase corticosterone responses to acute and continuous stressors and at the same time induce a state of glucocorticoid resistance in adult female mice [59]. Interestingly, another study found that a social interaction with a novel conspecific was not sufficient to induce higher levels of corticosterone in perinatally fluoxetine exposed rats [30], but they still found increased levels of corticosteroid-binding globulin [30, 60]. Together, this suggests that the HPA response is indeed modified after perinatal SSRI exposure. Although not investigated in our study, this altered HPA response to stress could underlie the differences between CTR- and FLX-females found in our study on prosocial behavior.

As a matter of fact, Ben-Ami Bartal et al. found an inverted U-shape effect of stress and another form of prosocial behavior: helping behavior. Both low levels of negative arousal (by midazolam treatment, a benzodiazepine anxiolytic acting on the HPA axis) *and* high corticosterone responses resulted in impairment of helping behavior in rats [61]. We suggested before that our aggressive encounters might not have been intense enough to trigger a strong stressful response. We hypothesized that this might underlie the lack of prosocial behavior in CTR-male rats. But the observation that high levels of stress also impair prosocial behavior, might be an explanation for the lack of prosocial behavior in FLX-rats, since they seem to have increases HPA responses to stress. In line with another study showing that prosocial behavior was negatively affected by HPA activity [62], Ben-Ami Bartal et al. concluded that the HPA reactivity results in antagonistic effects on helping behavior [61], because they might just get “too afraid to help” [63]. In fact, individuals with the short allele polymorphism of the serotonin

transporter gene regulatory region (5-HTTLPR) have also higher HPA reactivity [64] *and* lower prosocial tendencies [63]. Again, this suggests a clear relationship between serotonin, the HPA axis and prosocial behavior. In the case of our study, the stress caused by witnessing an aggressive encounter could be severe enough to evoke a different HPA reactivity in FLX-females compared to CTR-females. This could result in the impairment of prosocial behavior.

4.3 An additional stressor and prosocial behavior.

The explanation of the above on high levels of stress impairing prosocial behavior might also explain the lack of prosocial behavior found in CTR-females after the exposure to white-noise. While witnessing an aggressive encounter at baseline results in prosocial behavior because it caused only moderate levels of stress in the third-party bystanders, the exposure to white-noise could have induced higher levels of stress which subsequently block the display of prosocial behavior in the post-stress condition. In the case of FLX-females, the additional stressor even impaired the prosocial behavior more than before the white-noise. This finding was in line with our previous findings in terms of social behavior in which FLX-females switched from resting socially to more solitary after a stressful event [31]. In the current study, this effect was also seen in CTR-females and CTR-males.

Previously, we have found that the exposure to white-noise induces self-grooming behavior in FLX-males which was hypothesized to be due to alterations in stress-coping behavior [31]. In the current study, we do not observe elevated levels of self-grooming in CTR- and FLX-males after witnessing aggressive encounters in the pre- and post-stress conditions. An explanation can be found in that the social stressor in the current experiment could have been too light and short lasting to induce self-grooming. In addition, the behavior was observed immediately after the white-noise exposure in the previous study, while in the current study we

observed the behavior after the aggressive encounters, which occurred later on the day. Unpublished data revealed that the increased self-grooming behavior of FLX-males slowly attenuated over time (measured up to 3 hours after the white-noise), with a large variability between individuals. Thus, we conclude that an additional stressor did not affect the behavior of CTR- and FLX-rats in general after aggressive encounters. However, it did affect prosocial behavior of CTR-females, which confirms the hypothesis that an increased HPA reactivity impairs the motivation to show prosocial behavior.

5. Conclusion

In summary, we conclude in our test set-up that female rats are able to show prosocial behavior in response to witnessing aggressive encounters between conspecifics in a seminatural environment. We suggest that this prosocial behavior is most likely consolation behavior, but more research would be needed to confirm this. Male rats, on the other hand, did not show prosocial behavior in our test setting, suggesting that either they do not show consolation behavior, or our test set-up is not sufficient to detect this behavior in males. In addition, we showed that perinatal fluoxetine exposure impairs the display of prosocial behavior in female rats. We hypothesize that this is caused by an increased HPA reactivity, since an additional stressor (exposure to white-noise) also disrupts the prosocial behavior seen in CTR-females. Further research in the HPA reactivity is necessary to confirm this hypothesis

The effects of perinatal fluoxetine exposure on prosocial behavior could provide additional evidence that SSRI treatment during pregnancy could contribute to the risk for social impairments in the offspring. In conclusion, the experimental set-up used in this study may be of great help for the study of psychiatric disorders, in particular to the aspect of prosocial behavior.

6. Acknowledgments

Financial support was received from Helse Nord #PFP1295-16, Norway. DJH was supported by the KNAW ter Meulen travel grant, the Netherlands.

We also would like to thank Patty Huijgens for the rat images, and Aslaug Angelsen for the unpublished data on self-grooming. In addition, we thank Ragnhild Osnes, Carina Sørensen, Nina Løvhaug, Katrine Harjo, and Remi Osnes for their excellent care of the animals.

7. References

[1] Alwan, S., Reefhuis, J., Rasmussen, S. A., Friedman, J. M., National Birth Defects Prevention, S. Patterns of antidepressant medication use among pregnant women in a united states population. Journal of clinical pharmacology. 2011,51:264-70.

[2] Ververs, T., Kaasenbrood, H., Visser, G., Schobben, F., de Jong-van den Berg, L., Egberts, T. Prevalence and patterns of antidepressant drug use during pregnancy. European journal of clinical pharmacology. 2006,62:863-70.

[3] Kristensen, J. H., Ilett, K. F., Hackett, L. P., Yapp, P., Paech, M., Begg, E. J. Distribution and excretion of fluoxetine and norfluoxetine in human milk. Br J Clin Pharmacol. 1999,48:521-7.

[4] Rampono, J., Proud, S., Hackett, L. P., Kristensen, J. H., Ilett, K. F. A pilot study of newer antidepressant concentrations in cord and maternal serum and possible effects in the neonate. Int J Neuropsychopharmacol. 2004,7:329-34.

[5] Velasquez, J. C., Zhao, Q., Chan, Y., Galindo, L. C. M., Simasotchi, C., Wu, D., et al. In utero exposure to citalopram mitigates maternal stress effects on fetal brain development. ACS chemical neuroscience. 2019,10:3307-17.

[6] Homberg, J. R., Schubert, D., Gaspar, P. New perspectives on the neurodevelopmental effects of ssris. Trends in pharmacological sciences. 2010,31:60-5.

[7] Azmitia, E. C. Modern views on an ancient chemical: Serotonin effects on cell proliferation, maturation, and apoptosis. Brain Res Bull. 2001,56:413-24.

[8] Gaspar, P., Cases, O., Maroteaux, L. The developmental role of serotonin: News from mouse molecular genetics. Nature reviews. Neuroscience. 2003,4:1002-12.

[9] Lesch, K. P., Mossner, R. Genetically driven variation in serotonin uptake: Is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biological psychiatry. 1998,44:179-92.

[10] Weikum, W. M., Mayes, L. C., Grunau, R. E., Brain, U., Oberlander, T. F. The impact of prenatal serotonin reuptake inhibitor (sri) antidepressant exposure and maternal mood on mother-infant interactions at 3 months of age. Infant behavior & development. 2013,36:485-93.

[11] Brandlistuen, R. E., Ystrom, E., Eberhard-Gran, M., Nulman, I., Koren, G., Nordeng, H. Behavioural effects of fetal antidepressant exposure in a norwegian cohort of discordant siblings. International journal of epidemiology. 2015.

[12] Oberlander, T. F., Papsdorf, M., Brain, U. M., Misri, S., Ross, C., Grunau, R. E. Prenatal effects of selective serotonin reuptake inhibitor antidepressants, serotonin transporter promoter genotype (slc6a4), and maternal mood on child behavior at 3 years of age. Archives of pediatrics & adolescent medicine. 2010,164:444-51.

[13] Harrington, R. A., Lee, L. C., Crum, R. M., Zimmerman, A. W., Hertz-Picciotto, I. Prenatal ssri use and offspring with autism spectrum disorder or developmental delay. Pediatrics. 2014,133:e1241-8.

[14] Rai, D., Lee, B. K., Dalman, C., Golding, J., Lewis, G., Magnusson, C. Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: Population based case-control study. Bmj. 2013,346:f2059.

[15] Boukhris, T., Sheehy, O., Mottron, L., Berard, A. Antidepressant use during pregnancy and the risk of autism spectrum disorder in children. Jama Pediatrics. 2016,170:117-24.

[16] Croen, L. A., Grether, J. K., Yoshida, C. K., Odouli, R., Hendrick, V. Antidepressant use during pregnancy and childhood autism spectrum disorders. Archives of general psychiatry. 2011,68:1104-12.

[17] El Marroun, H., White, T. J., van der Knaap, N. J., Homberg, J. R., Fernandez, G., Schoemaker, N. K., et al. Prenatal exposure to selective serotonin reuptake inhibitors and social responsiveness symptoms of autism: Population-based study of young children. The British journal of psychiatry : the journal of mental science. 2014,205:95-102.

[18] Hagberg, K. W., Robijn, A. L., Jick, S. Maternal depression and antidepressant use during pregnancy and the risk of autism spectrum disorder in offspring. Clin Epidemiol. 2018,10:1599-612.

[19] Hviid, A., Melbye, M., Pasternak, B. Use of selective serotonin reuptake inhibitors during pregnancy and risk of autism. N Engl J Med. 2013,369:2406-15.

[20] Goodman, S. H. Depression in mothers. Annu Rev Clin Psychol. 2007,3:107-35.

[21] Dunkel Schetter, C. Psychological science on pregnancy: Stress processes, biopsychosocial models, and emerging research issues. Annual review of psychology. 2011,62:531-58.

[22] El Marroun, H., White, T., Verhulst, F. C., Tiemeier, H. Maternal use of antidepressant or anxiolytic medication during pregnancy and childhood neurodevelopmental outcomes: A systematic review. Eur Child Adolesc Psychiatry. 2014,23:973-92.

[23] Murray, L., Arteche, A., Fearon, P., Halligan, S., Goodyer, I., Cooper, P. Maternal postnatal depression and the development of depression in offspring up to 16 years of age. J Am Acad Child Adolesc Psychiatry. 2011,50:460-70.

[24] Brown, H. K., Hussain-Shamsy, N., Lunsky, Y., Dennis, C. E., Vigod, S. N. The association between antenatal exposure to selective serotonin reuptake inhibitors and autism: A systematic review and meta-analysis. The Journal of clinical psychiatry. 2017,78:e48-e58.

[25] Houwing, D. J., Staal, L., Swart, J. M., Ramsteijn, A. S., Wohr, M., de Boer, S. F., et al. Subjecting dams to early life stress and perinatal fluoxetine treatment differentially alters social behavior in young and adult rat offspring. Frontiers in neuroscience. 2019,13:229.

[26] Khatri, N., Simpson, K. L., Lin, R. C., Paul, I. A. Lasting neurobehavioral abnormalities in rats after neonatal activation of serotonin 1a and 1b receptors: Possible mechanisms for serotonin dysfunction in autistic spectrum disorders. Psychopharmacology (Berl). 2014,231:1191-200.

[27] Olivier, J. D., Valles, A., van Heesch, F., Afrasiab-Middelman, A., Roelofs, J. J., Jonkers, M., et al. Fluoxetine administration to pregnant rats increases anxiety-related behavior in the offspring. Psychopharmacology (Berl). 2011,217:419-32.

[28] Rodriguez-Porcel, F., Green, D., Khatri, N., Harris, S. S., May, W. L., Lin, R. C., et al. Neonatal exposure of rats to antidepressants affects behavioral reactions to novelty and social interactions in a manner analogous to autistic spectrum disorders. Anatomical record. 2011,294:1726-35.

[29] Simpson, K. L., Weaver, K. J., de Villers-Sidani, E., Lu, J. Y., Cai, Z., Pang, Y., et al. Perinatal antidepressant exposure alters cortical network function in rodents. Proc Natl Acad Sci U S A. 2011,108:18465-70.

[30] Gemmel, M., Hazlett, M., Bogi, E., De Lacalle, S., Hill, L. A., Kokras, N., et al. Perinatal fluoxetine effects on social play, the hpa system, and hippocampal plasticity in pre-adolescent male and female rats: Interactions with pre-gestational maternal stress. Psychoneuroendocrinology. 2017,84:159-71.

[31] Houwing, D. J., Heijkoop, R., Olivier, J. D. A., Snoeren, E. M. S. Perinatal fluoxetine exposure changes social and stress-coping behavior in adult rats housed in a seminatural environment. Neuropharmacology. 2019,151:84-97.

[32] Ko, M. C., Lee, L. J., Li, Y., Lee, L. J. Long-term consequences of neonatal fluoxetine exposure in adult rats. Dev Neurobiol. 2014,74:1038-51.

[33] Gemmel, M., De Lacalle, S., Mort, S. C., Hill, L. A., Charlier, T. D., Pawluski, J. L. Perinatal fluoxetine has enduring sexually differentiated effects on neurobehavioral outcomes related to social behaviors. Neuropharmacology. 2019,144:70-81.

[34] Zimmerberg, B., Germeyan, S. C. Effects of neonatal fluoxetine exposure on behavior across development in rats selectively bred for an infantile affective trait. Dev Psychobiol. 2015,57:141-52.

[35] Eisenberg, N., Miller, P. A. The relation of empathy to prosocial and related behaviors. Psychol Bull. 1987,101:91-119.

[36] de Waal, F. B. M., Vanroosmalen, A. Reconciliation and consolation among chimpanzees. Behav Ecol Sociobiol. 1979,5:55-66.

[37] Clay, Z., de Waal, F. B. Bonobos respond to distress in others: Consolation across the age spectrum. PLoS One. 2013,8:e55206.

[38] Cools, A. K. A., Van Hout, A. J. M., Nelissen, M. H. J. Canine reconciliation and third-party-initiated postconflict affiliation: Do peacemaking social mechanisms in dogs rival those of higher primates? Ethology. 2008,114:53-63.

[39] Palagi, E., Cordoni, G. Postconflict third-party affiliation in canis lupus: Do wolves share similarities with the great apes? Anim Behav. 2009,78:979-86.

[40] Plotnik, J. M., de Waal, F. B. M. Asian elephants (*elephas maximus*) reassure others in distress. Peerj. 2014,2.

[41] Seed, A. M., Clayton, N. S., Emery, N. J. Postconflict third-party affiliation in rooks, *corvus frugilegus*. Current biology : CB. 2007,17:152-8.

[42] Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B., Young, L. J. Oxytocin-dependent consolation behavior in rodents. Science. 2016,351:375-8.

[43] Ben-Ami Bartal, I., Decety, J., Mason, P. Empathy and pro-social behavior in rats. Science. 2011,334:1427-30.

[44] Bartal, I. B., Rodgers, D. A., Sarria, M. S. B., Decety, J., Mason, P. Pro-social behavior in rats is modulated by social experience. Elife. 2014,3.

[45] Sato, N., Tan, L., Tate, K., Okada, M. Rats demonstrate helping behavior toward a soaked conspecific. Animal cognition. 2015,18:1039-47.

[46] Marquez, C., Rennie, S. M., Costa, D. F., Moita, M. A. Prosocial choice in rats depends on food-seeking behavior displayed by recipients. Current biology : CB. 2015,25:1736-45.

[47] Lundmark, J., Reis, M., Bengtsson, F. Serum concentrations of fluoxetine in the clinical treatment setting. Ther Drug Monit. 2001,23:139-47.

[48] Chu, X., Agmo, A. Sociosexual behaviours in cycling, intact female rats (*rattus norvegicus*) housed in a seminatural environment. Behaviour. 2014,151:1143-84.

[49] Snoeren, E. M., Antonio-Cabrera, E., Spiteri, T., Musatov, S., Ogawa, S., Pfaff, D. W., et al. Role of oestrogen alpha receptors in sociosexual behaviour in female rats housed in a seminatural environment. J Neuroendocrinol. 2015,27:803-18.

[50] Knapska, E., Mikosz, M., Werka, T., Maren, S. Social modulation of learning in rats. Learning & memory. 2010,17:35-42.

[51] Fraser, O. N., Stahl, D., Aureli, F. Stress reduction through consolation in chimpanzees. Proc Natl Acad Sci U S A. 2008,105:8557-62.

[52] Boccia, M. L., Reite, M., Laudenslager, M. On the physiology of grooming in a pigtail macaque. Physiol Behav. 1989,45:667-70.

[53] Aureli, F., Preston, S. D., de Waal, F. B. Heart rate responses to social interactions in free-moving rhesus macaques (*macaca mulatta*): A pilot study. J Comp Psychol. 1999,113:59-65.

[54] Feh, C., Demazieres, J. Grooming at a preferred site reduces heart-rate in horses. Anim Behav. 1993,46:1191-4.

[55] Meerlo, P., Overkamp, G. J., Daan, S., Hoofdakker, R. H., Koolhaas, J. M. Changes in behaviour and body weight following a single or double social defeat in rats. Stress (Amsterdam, Netherlands). 1996,1:21-32.

[56] Meerlo, P., Overkamp, G. J. F., Benning, M. A., Koolhaas, J. M., Hoofdakker, R. H. Long-term changes in open field behaviour following a single social defeat in rats can be reversed by sleep deprivation. Physiology & behavior. 1996,60:115-9.

[57] Romero, T., de Waal, F. B. Chimpanzee (*pan troglodytes*) consolation: Third-party identity as a window on possible function. J Comp Psychol. 2010,124:278-86.

[58] Langford, D. J., Tuttle, A. H., Brown, K., Deschenes, S., Fischer, D. B., Mutso, A., et al. Social approach to pain in laboratory mice. Social neuroscience. 2010,5:163-70.

[59] Avitsur, R., Grinshpahet, R., Goren, N., Weinstein, I., Kirshenboim, O., Chlebowski, N. Prenatal ssri alters the hormonal and behavioral responses to stress in female mice: Possible role for glucocorticoid resistance. Horm Behav. 2016,84:41-9.

[60] Pawluski, J. L., Rayen, I., Niessen, N. A., Kristensen, S., van Donkelaar, E. L., Balthazart, J., et al. Developmental fluoxetine exposure differentially alters central and peripheral measures of the hpa system in adolescent male and female offspring. Neuroscience. 2012,220:131-41.

[61] Ben-Ami Bartal, I., Shan, H., Molasky, N. M., Murray, T. M., Williams, J. Z., Decety, J., et al. Anxiolytic treatment impairs helping behavior in rats. Front Psychol. 2016,7:850.

[62] Batson, C. D., Fultz, J., Schoenrade, P. A. Distress and empathy: Two qualitatively distinct vicarious emotions with different motivational consequences. Journal of personality. 1987,55:19-39.

[63] Stoltenberg, S. F., Christ, C. C., Carlo, G. Afraid to help: Social anxiety partially mediates the association between 5-httlpr triallelic genotype and prosocial behavior. Social neuroscience. 2013,8:400-6.

[64] Gotlib, I. H., Joormann, J., Minor, K. L., Hallmayer, J. Hpa axis reactivity: A mechanism underlying the associations among 5-httlpr, stress, and depression. Biological psychiatry. 2008,63:847-51.