



Effects of Selective Serotonin Reuptake Inhibitors Exposure in Stressed Pregnant Mice on the Anxiety Behavior of the Offspring

(Efek Pemberian SSRIs Prenatal pada Perilaku Kecemasan Offspring dari Model Maternal Stres)

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ABSTRACT

Background: Stress hurts problem-solving abilities, while anxiety disorders and depression are associated with unpleasant feelings and impaired daily functioning. Open field test (OFT) is used to assess anxiety-like behavior in animals by observing their exploratory behavior. Pregnant women are vulnerable to stress, which increases the risk of premature birth and low birth weight. Stress during pregnancy can also lead to preeclampsia and behavioural problems in newborns. Selective serotonin reuptake inhibitors (SSRIs), commonly used antidepressants during pregnancy, help manage mental disorders by increasing serotonin levels and regulating the endocrine system. They reduce glucocorticoid levels, thereby alleviating anxiety and stress in pregnant women. **Objectives:** This research investigates the correlation between the administration of SSRIs to stress-induced expectant mothers, the manifestation of depression-like behaviour, and the expression of glucocorticoid receptors in the offspring's hippocampus. **Material and Methods:** This study included four mouse groups: control without stress, control with offspring stress, stress model with footshock on dams and offspring, and stress with fluvoxamine treatment. Depressive and stress-related behaviors were measured using OFT. **Results:** OFT was used to assess the behaviour of offspring mice. Significant differences were observed in the number of crossings in the centre area and behaviour. The SSRI treatment showed potential anxiolytic effects, while stress led to reduced behaviour. These findings contribute to understanding anxiety-related responses and the effects of stress and SSRI treatment in animal models. **Conclusions:** OFT revealed significant differences in anxiety behaviour, specifically in the number of crossing centres, the centre area, and behaviour. SSRI treatment exhibited anxiolytic effects, while stress-decreased behaviour findings enhanced our understanding of anxiety responses and the impact of stress and SSRI treatment in animal models.



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INTRODUCTION

Stress, characterised by discomfort and tension resulting from human-environment interaction, hurts problem-solving abilities (Wahed et al, 2017). Anxiety disorders, categorised as mental disorders, entail unpleasant anxiety or worry about future events or fear in response to current events (Lovin et al, 2019). Additionally, depression is associated with impaired daily functioning and severe health conditions, manifesting symptoms of sadness, frustration, guilt, insensitivity, and loss of interest (Wahed et al, 2017; Mirzaei et al, 2019).

The open field test is a widely used behavioural assay that involves placing animals in an empty arena and monitoring their exploratory behaviour, providing insights into their emotional state and anxiety levels. This study aims to examine the influence of the open field test on animal anxiety-like behaviour, which is crucial for assessing anxiety disorders and developing potential interventions (La-Vu et al, 2020). The test is conducted in a square or rectangular plexiglas arena divided by marked zones or gridlines. It starts by placing a mouse in the centre and allowing it to explore the environment freely. Parameters measured include locomotor activity, reflecting general activity levels and exploratory behaviour. Centre zone activity indicates anxiety-like behaviour, as decreased time spent in the central area suggests avoidance of open spaces. Peripheral zone activity measures time near the walls, potentially indicating higher anxiety levels. Line crossings represent exploration and locomotor activity, while rearings indicate vertical exploration and increased arousal (Seibenhener & Wooten, 2015). Analysing these parameters provides valuable insights into the mice's emotional state and anxiety levels, aiding in assessing anxiety disorders and potential interventions (Sturman et al, 2018; Zimcikova et al, 2017).

Pregnant women are susceptible to stress, heightening the risk of premature birth and low birth weight for infants. Furthermore, stress during pregnancy contributes to the development of preeclampsia, potentially resulting in fetal death (Acosta et al., 2020). It is noteworthy that stress during pregnancy affects both mothers and newborns, as studies report the emergence of behavioural problems in newborns, including autism spectrum disorders, anxiety, schizophrenia, and other mental disorders (Fukui et al., 2023; Hagberg et al., 2018; Glover, 2015).

SSRIs, commonly used antidepressants during pregnancy, are beneficial in managing mental disorders such as depression, anxiety, and stress in both mothers and fetuses (Anderson et al., 2020). These medications work by inhibiting serotonin reuptake in the synaptic space, increasing serotonin concentration in the postsynaptic nerve terminal membrane (Edinoff et al., 2021). SSRIs can also affect the endocrine system of individuals with stress. By reducing glucocorticoid levels, SSRIs enhance the expression and function of the glucocorticoid receptor (GR) in the hippocampus, restoring negative

feedback by cortisol and inhibiting CRH secretion in the hypothalamus (PVN) (Domingues et al., 2023). This mechanism helps regulate the increased activity of the HPA axis, thereby aiding mothers with anxiety or stress (Dutton et al., 2022; Stafford et al., 2020; Sheng et al., 2021; Kageyama et al., 2021).

MATERIAL AND METHODS

Materials

The materials were fluvoxamine® (FUJIFILM Wako Pure Chemical) (4 mg), aquabidest (technical grade), Saline 0.9%, and apparatus open field test. The animals used were female and male ddY strain mice (*Mus musculus*). The certificate for ethical clearance was 2.KEH.061.04.2023 (Indonesia, Airlangga University Animal Care and Use Committee (ACUC)).

Methods

Animal Adaptation and Grouping

The experimental mice were acclimated for one week in individual cages (20 cm x 20 cm x 15 cm) covered with a wire mesh. They were maintained under controlled environmental conditions with a consistent food supply, proper ventilation, and a 12-hour light-dark cycle. After random allocation into four groups, each consisting of 8 female and eight male mice, the pregnant mice underwent specific treatments. Groups 1 and 2 served as control dams and received normal saline and feed. At the same time, Group 3 comprised stressed dams without treatment receiving saline, and Group 4 involved stressed dams with treatment receiving SSRIs and saline for 19-21 days. Subsequently, the offspring of these mice, totalling 5-7 individuals, were raised for 30 days and subjected to behavioural and molecular testing. The offspring were categorised into four groups based on the treatment received by their respective dams, including control dams without stress induction (NN), control dams with stress induction (SS), stressed dams without treatment (NS), and stressed dams with SSRIs treatment (SSRI).

Stress Model Development

Stress induction was conducted for approximately ten days. Each group consisted of 2 female mice, and SSRIs were administered on day 7 of stress induction. Behavioural evaluations were performed on approximately day 51 for the offspring of stressed dams, explicitly using the Open Field Test (OFT). Each animal underwent the OFT only once.

Animals Breeding

A male and female mouse were housed in a cage for mating. After mating, a vaginal plug in the female mouse indicated day 0 of pregnancy. The male and female mice were separated, and the pregnant females were prepared for stress induction and SSRI treatment in Group 3.

Footshock Procedure

The footshock (FS) model was employed to induce physical stress in pregnant female mice and their offspring with modification. A box measuring approximately 24 cm x 29 cm x 40 cm was divided into nine compartments containing a mouse, with a metal plate underneath for electric conduction. The experimental mice were acclimated to the communication box for 3 minutes. The induction process involved delivering electric shocks (0.2 mA) from the device ten times at a 15-second interval (Dorofeikova et al., 2023). After the induction, the FS-induced mice were returned to their cages and subjected to daily stress induction for 5 consecutive days. Once the offspring mice were born, they were subjected to stress induction at 31 days for seven consecutive days, with 30 stress inductions performed at 15-second intervals.

Preparation of SSRIs

The female mice were randomly assigned to four groups: two control groups without depression induction, receiving 0.9% saline; one group with FS-induced stress receiving 0.9% saline; and one group with FS-induced stress receiving SSRIs (fluvoxamine) at a dose of 4 mg/kg (Millard et al., 2017) body weight along with 0.9% saline for approximately 6 days. The female mice in the group with FS-induced stress and SSRI treatment received SSRIs administered orally (PO). The female mice were then subjected to treatment until one day before the birth of the offspring.

Open Field Test

On day 39 of the experiment, the OFT was conducted. The offspring mice were placed in a box measuring 40 cm x 40 cm with a height of 30 cm. The box was divided into 16 squares, each measuring 10 cm x 10 cm, marked with lines. The offspring mice were positioned along the edge of the box, facing the walls, and were tested for 5 minutes. After the 5-minutes test, the mice were returned to their cages, and the apparatus was cleaned.

Data Analysis

The data obtained from the Open Field Test will be analysed using statistical analysis with GraphPad Prism 9.3 software. Normality tests will be conducted to determine the appropriate analysis method. The normality test results indicate that the data can be analysed parametrically ($p > 0.05$). To assess the significance of the experiment, One-Way ANOVA will be performed, followed by post hoc Tukey's test ($*p < 0.05$, $**p < 0.01$, and $***p < 0.001$).

RESULTS AND DISCUSSION

Open Field Test (OFT) is a tool used to assess anxiety and behavioural changes in research animals by allowing them to explore an enclosed area (Schulz et al., 2023). The research data from the Open Field Test (OFT), conducted as the second test on offspring mice, revealed significant differences. The parameters measured in the OFT were the number of crossings in the centre area, time spent in the centre, number of rearings, number of crossings, and ratio number of crossings in the centre area. Based on the research findings presented in Figure 1-6, significant differences were observed using one-way ANOVA analysis of anxiety-like behaviour data obtained from the OFT data of four test groups.

Number of Crossing in The Center Area and Time in the Center

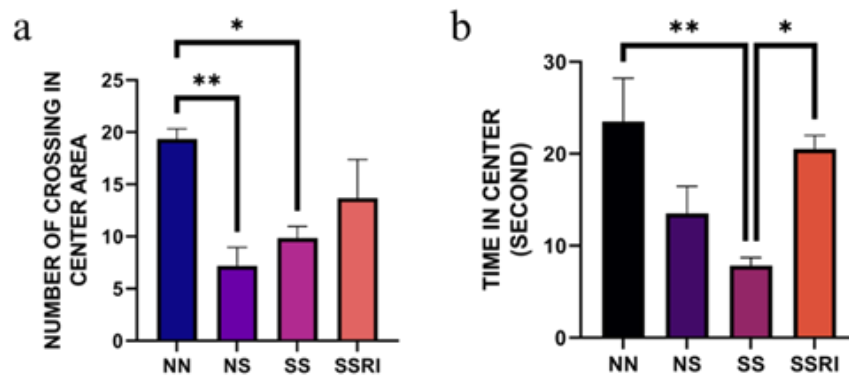


Figure 1. Results of Open Field Test (OFT) for assessing anxiety-like behaviour using various parameters, namely (a) the number of crossings in the centre area and (b) time spent in the centre area. Each bar represents the mean \pm SEM of 6 mice per group. Statistical significance is denoted by asterisks, with * $p < 0.05$ and ** $p < 0.01$. NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: Treatment of mother and offspring mice was induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

Table 1. Results of Time of Center in Open Field Test (OFT).

Offspring Mice	Treatment Group			
	NN	NS	SS	SSRI
1	15	12	10	20
2	19	26	6	21
3	17	4	7	19
4	14	10	10	15
5	35	14	9	22
6	41	15	5	26

*NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

Table 2. Results of Number of Crossing in The Center Area in Open Field Test (OFT).

Offspring Mice	Treatment Group			
	NN	NS	SS	SSRI
1	18	6	10	11
2	21	12	6	12
3	20	2	11	4
4	17	5	12	8
5	23	13	13	17
6	17	5	7	30

*NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

In Figure 1, the observed significant differences in crossings in the centre area can be attributed to the effects of different experimental conditions or treatments on the subject's behaviour. Both the SS group and NS group exhibited decreased crossings compared to the NN group, indicating the presence of a stressful or aversive stimulus that deterred exploration. Stress has been shown to impact exploratory behaviour and reduce willingness to explore novel environments (Kim & Kim, 2022). In contrast, the SSRI group did not show significant differences in the number of crossings compared to the SS group, suggesting that SSRI treatment did not significantly affect the locomotor activity or exploration behaviour. This aligns with SSRIs' primary mode of action, which targets neurotransmitter systems involved in mood regulation rather than locomotion (Hodge et al., 2015).

Regarding the time spent in the centre area, the SS group spent less time than the NN group, indicating that the stressful condition suppressed their willingness to spend time in the potentially aversive centre

region. Conversely, the SSRI group exhibited increased time spent in the centre area compared to the NN group, suggesting that the SSRI treatment attenuated the aversive or anxious response associated with the centre region, leading to increased exploration. Notably, the NS group did not show significant changes compared to the NN group, indicating that the absence of stress or experimental manipulation did not significantly influence their behaviour or exploration patterns in the centre area (Karamihalev et al., 2020). These interpretations are consistent with previous studies on stress and anxiety-related behaviour, demonstrating the impact of stress on exploration, locomotor activity, and time spent in specific regions of a testing apparatus (Johnson, A. et al., 2023; Petković and Chaudhury, 2022 Wardwell, et al., 2020).

Number of Crossing and Rearing

Table 3. Results of Number of Crossing in Open Field Test (OFT).

Offspring Mice	Treatment Group			
	NN	NS	SS	SSRI
1	131	93	108	102
2	109	105	90	92
3	121	93	128	88
4	134	97	111	97
5	130	106	120	100
6	93	94	99	122

*NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method.

SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

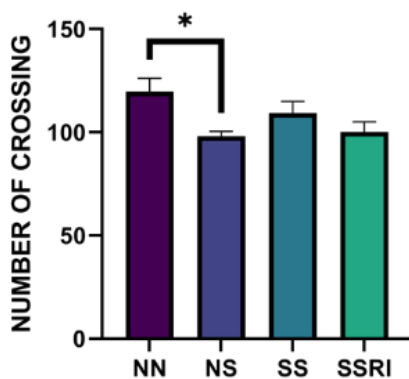


Figure 2. Results of Open Field Test (OFT) for assessing anxiety-like behaviour using various parameters, namely number of crossings. Each bar represents the mean \pm SEM of 6 mice per group. Statistical significance is denoted by asterisks, with $*p < 0.05$. NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

The number of rearings in Figure 2 did not show significant differences; thus, no further post hoc Tukey's analysis was conducted. However, the number of crossings in Figure 3 revealed statistically significant differences. The NS group displayed a significant decrease in crossings compared to the NN group. However, no significant differences were found when comparing the SS group with the NN group or the SSRI group with the SS group.

The lack of significant differences in the number of rearings suggests that this parameter may not be sensitive enough to detect variations in anxiety-like behaviour among the groups. Other factors, such as general locomotor activity or exploratory behaviour, might have influenced the number of rearings instead (Klein et al., 2022). Regarding the number of crossings, the significant decrease observed in the NS group compared to the NN group may indicate that the presence of a stressor led to reduced exploratory behaviour and increased anxiety-like responses (Tseitlin et al., 2023; Eraslan et al., 2023; da Costa et al., 2023). On the other hand, the non-significant differences between the SS group and the NN group, as well as between the SSRI group and the SS group, suggest that the SS condition or the SSRI treatment may not have had a significant impact on locomotor activity or exploration behaviour in this particular context (Sturman et al., 2018; Heinz et al., 2021). These interpretations align with previous research demonstrating the influence of stress and interventions on exploratory behaviour and anxiety-like responses in animal models (Tucker et al., 2022; Verbitsky et al., 2020; Becker et al., 2021).

Table 4. Results of Number of Rearing in Open Field Test (OFT).

Offspring Mice	Treatment Group			
	NN	NS	SS	SSRI
1	41	27	20	16
2	16	23	19	13
3	27	12	14	10
4	42	30	17	14
5	13	53	12	16
6	18	31	13	18

* NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

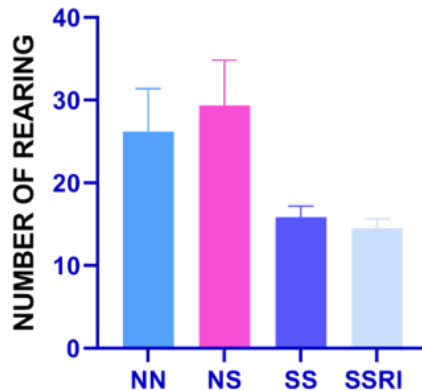


Figure 3. Results of Open Field Test (OFT) for assessing anxiety-like behaviour using various parameters, namely number of rearing. Each bar represents the mean \pm SEM of 6 mice per group. NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

Number of Grooming

Table 5. Results of Number of Grooming in Open Field Test (OFT).

Offspring Mice	Treatment Group			
	NN	NS	SS	SSRI
1	5	8	5	5
2	10	5	5	7
3	11	6	8	5
4	8	10	5	6
5	12	9	3	5
6	8	4	4	4

*NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

In Figure 4, significant differences were observed in the grooming parameter. The SS group exhibited a decrease in grooming compared to the NN group, while the SSRI group showed an increase compared to the NN group. However, no significant changes were found between the NS and NN groups. There were no significant differences between the SSRI group and the SS group. The significant decrease in grooming observed in the SS group compared to the NN group may indicate an alteration in self-care behaviours related to anxiety-like behaviour (Wang et al., 2023; León-Rodríguez et al., 2022). Grooming can be a self-soothing behaviour and can be affected by stress and anxiety. The decrease in grooming in the SS group suggests a potential increase in anxiety-like responses (Dreisoerner et al, 2021; Lim et al, 2023; Wardwell et al, 2020).

On the other hand, the increase in grooming observed in the SSRI group compared to the NN group suggests that the SSRI treatment might have had an anxiolytic effect, reducing anxiety-like behaviour and promoting self-care behaviours (Garakani et al., 2020; Bandelow et al., 2017; Nitzan et al., 2022; Heesbeen et al., 2023). The modulation of neurotransmitter systems involved in mood regulation by SSRIs could contribute to this effect (Spurny et al., 2021; Jiang et al., 2022; Alosaimi et al., 2022). The lack of significant changes in grooming in the stress group compared to the NN group suggests that the absence of stress or experimental manipulation did not significantly influence grooming behaviour (Kalueff et al., 2015; Zhang et al., 2022).

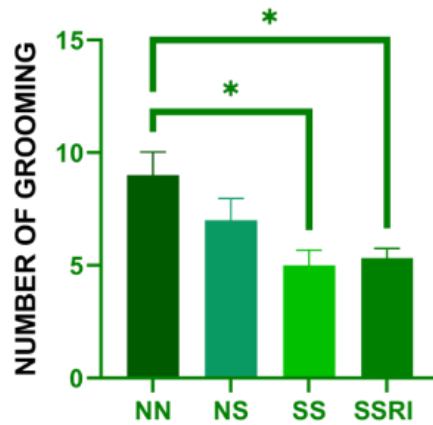


Figure 4. Results of Open Field Test (OFT) for assessing anxiety-like behaviour using various parameters, namely the number of grooming. Each bar represents the mean \pm SEM of 6 mice per group. Statistical significance is denoted by asterisks, with $*p < 0.05$. NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: Treatment of mother and offspring mice was induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

The ratio of the Number of Crossing in The Center Area

Table 6. Results of Ratio of Number of Crossings in The Center Area in Open Field Test (OFT).

Offspring Mice	Treatment Group			
	NN	NS	SS	SSRI
1	0,136	0,064	0,138	0,107
2	0,196	0,086	0,111	0,130
3	0,165	0,026	0,077	0,050
4	0,120	0,051	0,108	0,082
5	0,165	0,109	0,158	0,186
6	0,182	0,042	0,089	0,222

*NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

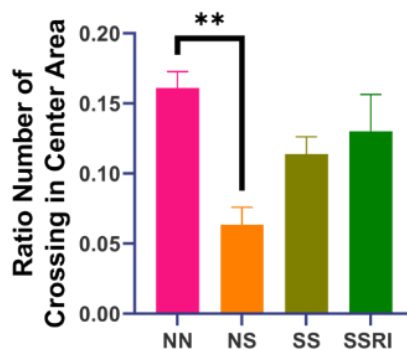


Figure 5. Results of Open Field Test (OFT) for assessing anxiety-like behaviour using various parameters, namely the ratio of crossings in the centre area. Each bar represents the mean \pm SEM of 6 mice per group. Statistical significance is denoted by asterisks, with $**p < 0.01$. NN: treatment of offspring mice without prenatal stress and footshock induction. NS: treatment of offspring mice with induced stress from mother mice that were not induced by stress. SS: treatment of mother and offspring mice induced by stress using a communication box electric shock device using the footshock method. SSRI: treatment of stress-induced mother mice and offspring mice using a communication box electric shock device using the footshock method, while the mother mice were given SSRI treatment (fluvoxamine) at 4 mg/kg BW.

The data in Figure 5 indicate a significant difference in the ratio of crossings in the centre area based on one-way ANOVA analysis. The stress group experienced a significant decrease compared to the Normal group. However, the maternal stress group did not show a significant increase or decrease compared to the Normal group. Furthermore, the SSRI group showed no significant difference compared to the maternal stress group. The significant decrease in the ratio of crossings in the centre area observed in the stress group compared to the Normal group may suggest heightened anxiety-like behavior or reduced exploratory behavior. Stress can influence the willingness to venture into novel or potentially aversive areas, leading to decreased crossings in the centre region.

The non-significant difference between the maternal stress group and the Normal group suggests that the maternal stress condition may not have significantly affected the ratio of crossings in the centre area. It is possible that the stressors experienced by the mothers did not result in an observable impact on the exploratory behaviour of their offspring. The absence of a significant difference between the SSRI and maternal stress groups indicates that the SSRI treatment did not significantly impact the ratio of crossings in the centre area in this context. This finding aligns with the mode of action of SSRIs, primarily targeting neurotransmitter systems involved in mood regulation rather than locomotion or exploration behaviour.

CONCLUSION

This study used the Open Field Test (OFT) to assess anxiety-like behaviour in offspring mice. Significant differences were observed in the number of crossings in the centre area and grooming behaviour. The SSRI treatment demonstrated potential anxiolytic effects, while stress led to reduced

exploratory behavior. These findings contribute to understanding anxiety-related responses and the effects of stress and SSRI treatment in animal models. Additionally, the maternal stress condition did not significantly affect the offspring's exploration behaviour, and the SSRI treatment did not significantly impact the behaviour in the context of the OFT. These results highlight the complex interplay between stress, SSRI treatment, and behaviour in animal models.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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