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ASSESSMENT OF SEROTONERGIC TRANSMISSION DURING SOCIAL INTERACTION IN MICE

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ABSTRACT

Social behavior deficits are characteristic features of several neuropsychiatric and neurodevelopmental disorders, including autism spectrum disorder, schizophrenia, and social anxiety disorder. Serotonin (5-hydroxytryptamine; 5-HT) plays a critical role in regulating social cognition and behavior; however, the specific contribution of serotonergic signaling to sociability remains incompletely understood. The present study investigated the role of serotonergic neurotransmission in social interaction behavior using the sociability test in Swiss albino mice. Mice were treated intraperitoneally with the serotonin precursor 5-hydroxy-L-tryptophan (5-HTP; 1, 5, 25, and 75 mg/kg), the serotonergic receptor antagonist metergoline (1, 5, and 10 mg/kg), or the serotonin-releasing agent fenfluramine (1, 5, and 10 mg/kg). Social behavior was assessed using the three-chamber sociability apparatus. Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc testing. Administration of 5-HTP produced a significant enhancement of sociability at 75 mg/kg [$F(4,35)=39.93$, $p<0.05$], whereas lower doses were ineffective. Metergoline significantly reduced social interaction [$F(2,15)=61.06$, $p<0.0001$], indicating the involvement of endogenous serotonergic activity in maintaining normal social behavior. Fenfluramine significantly altered social interaction behavior [$F(2,15)=47.16$, $p<0.0001$] and modified the effects of 5-HTP. Furthermore, fenfluramine significantly influenced 5-HTP-induced sociability, whereas metergoline failed to alter the effect of 5-HTP [$F(3,20)=51.16$, $p<0.0001$]. These findings demonstrate that serotonergic neurotransmission plays a crucial role in regulating social behavior in mice. Enhancement of central serotonin levels improves sociability, whereas serotonergic receptor modulation significantly influences social interaction. The serotonergic system may therefore represent a promising therapeutic target for social deficits associated with neuropsychiatric disorders.

KEYWORDS: Serotonin; 5-Hydroxy-L-tryptophan (5-HTP); Sociability; Social Interaction.

1. INTRODUCTION

Social behavior deficits are a hallmark feature of several neuropsychiatric and neurodevelopmental disorders, including autism spectrum disorder (ASD), schizophrenia, social anxiety disorder, major depressive disorder, and certain forms of dementia. Impairments in social interaction, communication, and social recognition significantly affect quality of life and functional outcomes in affected individuals (Duerler, Vollenweider et al. 2022). Despite advances in understanding the neurobiology of these disorders, effective pharmacological interventions specifically targeting social dysfunction remain limited, highlighting the need to identify the neurotransmitter systems involved in the regulation of social behavior. Current therapeutic approaches primarily focus on managing associated symptoms such as anxiety, depression, irritability, or psychosis rather than directly improving social deficits. Selective serotonin reuptake inhibitors (SSRIs), atypical antipsychotics, and anxiolytic agents are commonly prescribed in disorders characterized by impaired social functioning (Rodnyy, Kondaurova et al. 2024). Among these, serotonergic drugs have attracted considerable attention because serotonin (5-hydroxytryptamine; 5-HT) is a key neurotransmitter involved in emotional processing, social cognition, reward, mood regulation, and behavioural adaptation. Clinical and preclinical studies have demonstrated that modulation of serotonergic neurotransmission can influence social interaction, social preference, and social recognition behaviors (Duerler, Vollenweider et al. 2022, Rodnyy, Kondaurova et al. 2024).

Serotonin is synthesized from the essential amino acid tryptophan and is widely distributed throughout the central nervous system. Serotonergic neurons originating from the raphe nuclei project extensively to brain regions implicated in social behavior, including the prefrontal cortex, amygdala, hippocampus, nucleus accumbens, and hypothalamus. The actions of serotonin are mediated through multiple receptor subtypes (5-$HT_{1–5}$-HT_7), each contributing differently to neuronal signalling and behavioural responses. In addition, serotonin availability within the synaptic cleft is tightly regulated by the serotonin transporter (SERT), making serotonergic signalling a critical determinant of behavioural outcomes (Rodnyy, Kondaurova et al. 2024).

Accumulating evidence suggests that disturbances in serotonergic neurotransmission contribute to social behavior abnormalities. Altered serotonin levels, impaired serotonin transporter function, and dysregulation of specific serotonin receptor subtypes have been reported in several psychiatric disorders characterized by social deficits (Salvan, Fonseca et al. 2023). Experimental studies in rodents have shown that pharmacological enhancement of serotonin signalling can improve social interaction, whereas serotonin depletion or receptor dysfunction may impair sociability and social recognition. These findings indicate that the serotonergic system plays a crucial role in modulating social behavior and may represent a promising therapeutic target for disorders associated with social dysfunction (Jacobs, Maior et al. 2025).

Several well-established experimental models are used to investigate the neurobiological mechanisms underlying social behaviour (Li, Williams et al. 2021). Behavioural paradigms assessing sociability and social recognition offer valuable tools for evaluating the effects of pharmacological manipulation of neurotransmitter systems. Such models facilitate the identification of specific serotonergic pathways that regulate social interactions and provide insight into potential therapeutic strategies (Walsh, Christoffel et al. 2023). Based on the established involvement of serotonin in social cognition and behavior, it is hypothesized that modulation of serotonergic neurotransmission can significantly influence social interaction patterns in mice. Therefore, the present study was undertaken to investigate the role of the serotonergic (5-HT) system in regulating social interaction behavior in mice and to evaluate how alterations in

serotonin signalling and receptor activity affect sociability and social recognition.

2. MATERIALS AND METHODS

Drug and Chemicals

Serotonin agonist fenfluramine and serotonin antagonist metergoline or 5-HTP (5-hydroxy L-tryptophan hydrate was purchased from Sigma-Aldrich, USA. These drugs were dissolved in (dissolved in double distilled water) and administered through intraperitoneally. Required dose was administered intraperitonially (i.p.). The doses of all the drugs were calculated as the free base.

Animal

Swiss albino mice (25-28 g) of either sex. They were housed in acrylic cages (24X17X12 cm) under controlled environmental condition (22 ± 1ċ, 65 ± 5% RH), maintained at 12:12 h light/dark cycle (light on 08:20:00 hrs.). The Mice were fed with standard diet and water ad libitum. The experimental were designed and conducted in accordance with the ethical norms approved by Committee for the Control and Supervision of Experimental Animal (CCSEA) and Institutional Animal Ethics Committee of Institute SIRT-Pharmacy.

Sociability and Social Novelty Test

Social interaction and social novelty behaviors were assessed using the three-chamber social interaction apparatus with minor modifications of the method described by Moy et al. (2004) and Crawley (2007). Experiments were conducted under illumination of 300–330 lux.

The apparatus consisted of three interconnected Plexiglas chambers of equal dimensions. During the habituation phase, each test mouse was placed in the central chamber and allowed to explore for 5 min while access to the side chambers was blocked.

Experimental Design

Mice were randomly divided into five groups (n = 6 per group) and treated intraperitoneally (i.p.) as follows:

i. **Group I (Control group):** Mice were administered vehicle (10 mL/kg, intraperitoneally, i.p.).
ii. **Group II (5-HTP 1 mg/kg treated group):** Mice were administered 5-hydroxy-L-tryptophan (5-HTP) at a dose of 1 mg/kg body weight (i.p.).
iii. **Group III (5-HTP 5 mg/kg treated group):** Mice were administered 5-hydroxy-L-tryptophan (5-HTP) at a dose of 5 mg/kg body weight (i.p.).
iv. **Group IV (5-HTP 25 mg/kg treated group):** Mice were administered 5-hydroxy-L-tryptophan (5-HTP) at a dose of 25 mg/kg body weight (i.p.).
v. **Group V (5-HTP 75 mg/kg treated group):** Mice were administered 5-hydroxy-L-tryptophan (5-HTP) at a dose of 75 mg/kg body weight (i.p.).

Behavioral Assessment

Effect of 5-HTP or 5-hydroxy L-tryptophan on sociability test:

Separate group of mice (n=6) were treated intraperitoneally (i.p.) with vehicle (10 ml/kg) or 5-hydroxy L-tryptophan (1, 5, 25, 75 mg/kg). 30 minutes after i.p. administration of 5-hydroxy L-tryptophan reached a maximum effect 30 minutes after treatment in the mice. Therefore, in the present study we treated mice with 5-hydroxy L-tryptophan 30

minutes before the test and then after 20 min mice were subjected to sociability test apparatus for 10 min for the assessment of sociability and social novelty seeking behavior.

Effect of serotonin receptor antagonist on sociability test

Mice, in a separate group (n=6) were treated with) with either vehicle (10 ml/kg, i.p.) or serotonin receptor antagonist, metergoline (5, 10 mg/kg, i.p.) and 30 min there after animals received either vehicle (10 ml/kg, i.p.) or serotonin receptor antagonist. Then after 30 min mice were subjected to sociability test apparatus for 10 min for the assessment of sociability and social novelty seeking behaviour.

Effect of serotonin receptor agonist on sociability test

Separate group (n=6) were treated with either vehicle (10 ml/kg, i.p.) or serotonin receptor agonist, fenfluramine (5, 10 mg/kg, i.p.) and 30 min there after animals received either vehicle (10 ml/kg, i.p.) or serotonin receptor antagonist. Then after 30 min mice were subjected to sociability test apparatus for 10 min for the assessment of sociability and social novelty seeking behaviour.

Effect of 5-HT receptor antagonist or agonist on 5-hydroxy L-tryptophan induced effect on sociability test

Separate group (n=6) were treated with either vehicle (10 ml/kg, i.p.) or serotonin receptor antagonist, metergoline (5 mg/kg, i.p.) or serotonin receptor agonist, fenfluramine (5 mg/kg, i.p.) or 5-hydroxy L-tryptophan (75 mg/kg, i.p.). was administered to separate groups of mice (n=6), after 30 min of i.p. injection of either vehicle (10 ml/kg) or 5-hydroxy L-tryptophan (75 mg/kg, i.p.). Then after 30 min mice were subjected to sociability test apparatus for 10 min for the assessment of sociability and social novelty seeking behaviour.

Effect of serotonergic analogues on locomotor activity

Separate group (n=6) were treated with vehicle (10 ml/kg, i.p.) or serotonin receptor antagonist, metergoline (5, 10 mg/kg, i.p.) or serotonin receptor agonist, fenfluramine (5, 10 mg/kg, i.p.) or 5-hydroxy L-tryptophan (1, 5, 25, 75 mg/kg). was injected to different groups of mice at appropriate time period as described above. Then after 20 min mice were subjected to sociability test apparatus for 10 min for the assessment of sociability and social novelty seeking behaviour.

Statical Analysis

Data are expressed as mean ± SEM. Most data were analyzed using one-way analysis of variance (ANOVA). Repeated-measures ANOVA was used to analyzed socialization apparatus data and locomotor activity data from place conditioning trials. Newman Keuls or Tukey's multiple comparison was used as follow-up test and one-way analysis of variance. $P < 0.05$ was considered statistically significant and all data were collected using prism pad chart.

3. RESULTS

Effect of serotonin precursor 5-HTP or 5-hydroxy L-tryptophan in sociability test

One way analysis of variance (ANOVA) shows remarkable effect of 5-HTP treatment (25 and 75 mg/kg; i.p.) on sociability test of mice [$F(4, 35) = 39.93$, $P<0.05$ One way analysis of variance (ANOVA) followed by Newman-Keuls test indicates that 5-HTP (75 mg/kg; i.p.) significantly increase the sociability test of mice as compared to saline treated animal *($P<0.05$) However, 5-HTP in the dose 1 and 5 mg/kg; i.p. was devoid of any effect on sociability test *($P<0.05$).

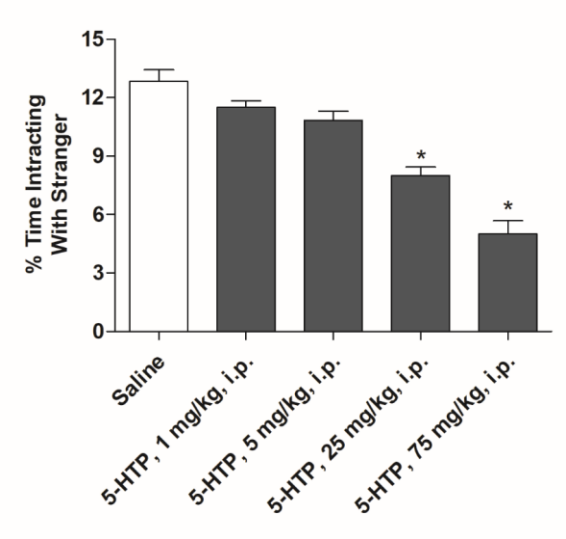

Figure 1: Effects of serotonin precursor 5-HTP on sociability test.

Separate group of mice (n=6) were treated intraperitoneally (i.p.) with vehicle (10 ml/kg) or 5-HTP (1, 5, 25, 75 mg/kg, i.p.). Half an hours after i.p. administration of 1, 5, 25, 75 mg/kg, i.p. of 5-HTP, each mouse was subjected to sociability test for 15 min. Each value represents the mean ± S.E.M. 6 animals. *<0.05 compared with vehicle-treated group (one-way analysis of variance (ANOVA) followed by Newman Keuls test). These results are shown in Figure 1.

7.2 Effects of 5-HT_1 receptor antagonist on sociability test.

One way analysis of variance (ANOVA) shows remarkable effects of 5-HT_1 receptor antagonist, metergoline (1, 5, 10 mg/kg, i. p.) treatment on sociability test of mice [F (2, 15) = 61.06, *P<0001]. One way analysis of variance (ANOVA) followed by Newman-Keuls test indicates that 5-HT_1 receptor antagonist, metergoline (1, 5, 10 mg/kg, i. p.) significantly reduced the sociability test of mice as compared to vehicle treated animals (*P<0001). These results are shown in Figure 2.

Mice were treated with a saline or $5HT_1$ receptor antagonist, metergoline (1, 5, 10 mg/kg, i. p.). Ten minutes thereafter mice were introduced to sociability test for 15 min and number of interactions was noted at the end of the test session. Each value shows the mean ± S.E.M. of 6 mice. P<0001 as compared to saline treated group (One Way ANOVA followed by Newman-Keuls test).

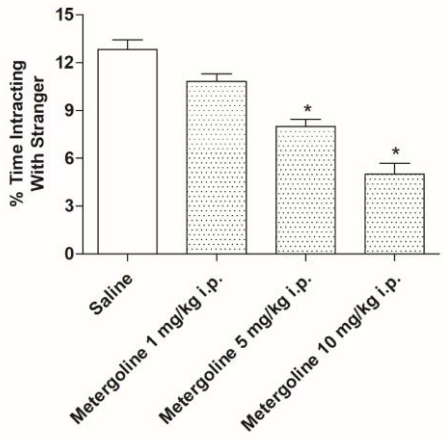

Figure 2: Effects of 5-HT_1 receptor antagonist on sociability test.

Effect of 5-HT$_1$ receptor agonist fenfluramine on sociability test:

One way analysis of variance (ANOVA) shows remarkable effects of fenfluramine treatment on sociability test of mice [F (2,15) =47.16, p<0001]. Post hoc Newman-Keuls test indicates that treatment of 5-HT$_1$ receptor agonist, Fenfluramine (1, 5, 10 mg/kg, i.p.) significantly potentiate decrease in social interaction (*p<0001). This result is represented in Figure 3.

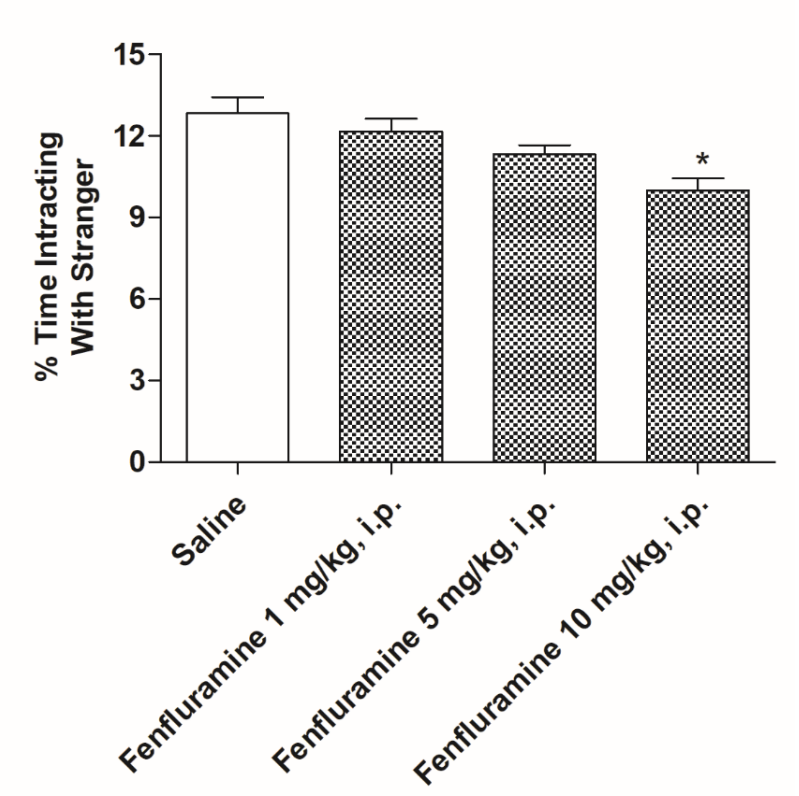

Figure 3: Effects of 5-HT$_1$ receptor agonist on sociability test.

Saline (10 ml/kg, i.p.) or Fenfluramine (2, 5, 10 mg/kg, i.p.) was administered to separate groups of mice (n=6) after 30 min of injection of either saline (10 ml/kg). Fifteen min thereafter each mouse was subjected to sociability test for 15 min. Each value represents the mean ± S.E.M. of 6 animals. *P<000.1 vs. saline treated group *p<000.1 (One way analysis of variance (ANOVA) followed by Newman-Keuls test).

Effect of Metergoline, Fenfluramine on 5-hydroxy L-tryptophan induced effect on sociability test

One way analysis of variance (ANOVA) shows remarkable effects of Metergoline, Fenfluramine treatment on 5-hydroxy L-tryptophan induced sociability test of mice [F (3,20) =51.16, p<0001]. Post hoc Newman-Keuls test indicates that treatment of 5-HT$_1$ receptor agonist, Fenfluramine (10 mg/kg, i.p.) significantly reversed 5-hydroxy L-tryptophan induced decrease in social interaction (*p<0001). However, 5-HT$_1$ receptor antagonist, Metergoline (10 mg/kg, i.p) failed to affect 5-hydroxy L-tryptophan induced effects on sociability test p<0.05. This result is represented in Figure 4.

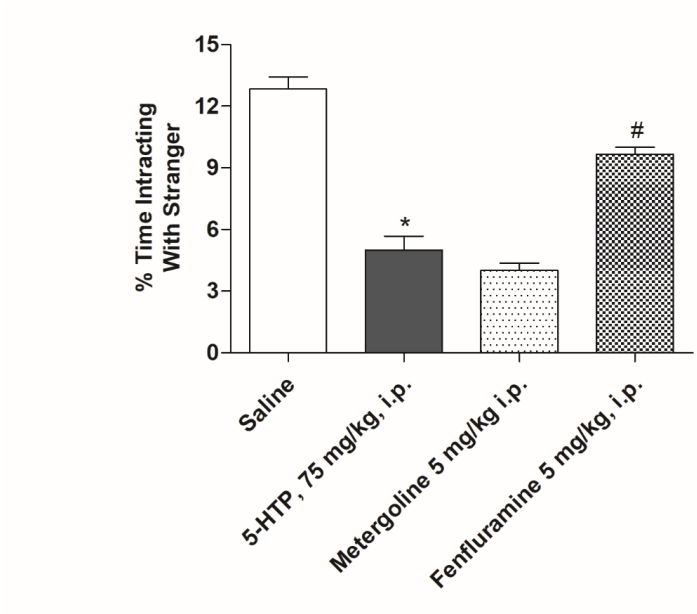

Figure 4: Effects of Metergoline, Fenfluramine on 5-hydroxy L-tryptophan induced interaction in sociability test.

Saline (10 ml/kg, i.p.) or Fenfluramine (10 mg/kg, i.p.) or Metergoline (10 mg/kg, i.p.) was administered to separate groups of mice (n=6) after 30 min of injection of either saline (10 ml/kg) and 5-hydroxy L-tryptophan (75 mg/kg).

Fifteen min thereafter each mouse was subjected to sociability test for 15 min. Each value represents the mean ± S.E.M. of 6 animals. *P<000.1 vs. saline treated group #p<000.1 vs. saline + 5-hydroxy L-tryptophan treated group. (One way analysis of variance (ANOVA) followed by Newman-Keuls test).

4. DISCUSSION

The present study investigated the role of serotonergic neurotransmission in the regulation of social behavior using the sociability test in mice. Administration of the serotonin precursor 5-hydroxy-L-tryptophan (5-HTP) significantly enhanced social interaction at a dose of 75 mg/kg, suggesting that increased central serotonin levels facilitate sociability. Social isolation during adolescence has been reported to produce long-lasting alterations in emotional behavior and brain regions associated with anxiety and stress responses in adulthood (Butler, Karkhanis et al. 2016).

Therefore, modulation of serotonergic neurotransmission may represent an important mechanism underlying social behavior.

To examine the contribution of endogenous serotonin to social interaction, the effect of the serotonin precursor 5-HTP was evaluated in the sociability test. The results demonstrated that administration of 5-HTP (75 mg/kg, i.p.) significantly increased social interaction in mice. This finding is consistent with previous reports showing that systemic administration of 5-HTP enhances serotonin synthesis because aromatic L-amino acid decarboxylase is not saturated under physiological conditions (Marchesi, Macchi et al. 1972). Furthermore, Fonseca et al. (2023) reported that 5-HTP administration significantly elevates whole-brain serotonin concentrations in mice. Thus, the enhanced sociability observed in the present study is likely attributable to increased central serotonergic transmission (Salvan, Fonseca et al. 2023). These findings suggest that pharmacological modulation of endogenous serotonin levels may represent a potential strategy for improving social behavior deficits. To further characterize the serotonergic mechanisms involved,

the effects of the serotonergic receptor antagonist metergoline were examined. Metergoline significantly reduced social interaction, indicating that endogenous serotonergic activity contributes to the maintenance of normal social behavior.

Moreover, the attenuation of sociability following serotonergic receptor blockade supports the hypothesis that serotonin plays a facilitatory role in social interaction. Previous studies have similarly demonstrated that alterations in central serotonin levels markedly influence behavioral responses (Huq, Warner et al. 2021).

The role of serotonergic receptors was further explored using fenfluramine, a serotonin-releasing agent that enhances serotonergic neurotransmission. Fenfluramine significantly modified social interaction behavior and altered the effects produced by 5-HTP. These findings suggest that enhanced serotonergic activity may influence sociability through specific serotonin receptor subtypes. The observation that serotonergic receptor modulation affects social interaction supports the involvement of serotonin-dependent signaling pathways in the regulation of social behavior (Tupal and Faingold 2019). Evidence from previous studies indicates that serotonergic receptors, particularly 5-HT_2A and 5-HT_2C receptors, are involved in emotional and anxiety-related behaviors (Pędzich, Rubens et al. 2022). Increased serotonin release following local infusion of serotonin into the suprachiasmatic nucleus has been shown to be attenuated by methysergide, a serotonergic receptor antagonist. Furthermore, meta-chlorophenylpiperazine (m-CPP), which acts primarily at 5-HT_2C receptors and to a lesser extent at 5-HT_1A and 5-HT_1D receptors, has been widely used as a pharmacological model for investigating anxiety-related and social behaviors (Zohar et al., 2000). Activation of these receptor subtypes has been associated with reductions in social interaction and increased anxiety-like responses.

Therefore, the effects observed following administration of 5-HTP may be attributed to increased central serotonin levels, which subsequently modulate serotonergic neurotransmission through specific receptor subtypes (Weinberg-Wolf, Fagan et al. 2018). The present findings support the involvement of serotonergic mechanisms in the regulation of social behavior and suggest that alterations in serotonin signaling can significantly influence sociability. However, the precise receptor subtype responsible for these effects remains to be elucidated. Future studies employing selective agonists and antagonists for individual serotonin receptor subtypes are warranted to clarify the underlying neuropharmacological mechanisms. Although the sociability test is a valuable behavioral model for assessing social interaction, some investigators have suggested that it may not clearly distinguish between anxiolytic and anti-compulsive drug effects. Therefore, complementary behavioral paradigms should be employed in future studies to further characterize the role of serotonergic neurotransmission in social behavior.

5. CONCLUSION

The present study demonstrates that serotonergic neurotransmission plays an important role in the regulation of social interaction and social novelty behavior in mice. Pharmacological modulation of the serotonergic system using 5-hydroxy-L-tryptophan (5-HTP), metergoline, and fenfluramine significantly influenced sociability, indicating the involvement of serotonin in social behavior. Importantly, these effects occurred without significant alterations in locomotor activity, suggesting a specific role of serotonin in social processes rather than general motor function.

Collectively, these findings enhance our understanding of the neuropharmacological mechanisms underlying social behavior and highlight the serotonergic system as a potential therapeutic target for social deficits associated with neuropsychiatric disorders. Further studies are warranted to investigate the long-term effects and receptor-specific mechanisms involved in serotonergic modulation of social behavior.

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