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Mitigation of addictive effects induced by lorazepam through concurrent administration of SSRI: Interplay of serotonin and dopamine in caudate and nucleus accumbens

Huma Ikram[1]*, Iqra Atique[1], Umaira M Moosa[1] and Darakhshan Jabeen Haleem[1,2]
[1]Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan
[2]Neuroscience Research Laboratory, Dr Panjwani Center for Molecular Medicine and Drug Research, ICCBS, University of Karachi, Karachi, Pakistan

Abstract: The present study aimed to assess the antidepressant profile of fluoxetine in the rats exhibiting lorazepam-induced abusive effects in place preference paradigm. Lorazepam, a benzodiazepine is commonly utilized for treating anxiety, panic attacks, status epilepticus, depressive disorders and sedation. Despite its therapeutic benefits, repeated lorazepam administration can lead to dependence, possibly involving heightened dopaminergic neurotransmission. Additionally, an important role is played by serotonergic system in anxiety and addiction pathophysiology and treatment. The study aimed to examine fluoxetine's impact on lorazepam-induced addiction, as fluoxetine, a selective serotonin reuptake inhibitor, enhances 5-HT availability by inhibiting its reuptake in neurons. Behavioral parameters, including growth rate, food intake, behaviors in forced swim test, open field, light dark box test, Skinner's box and conditioned place preference, were monitored in rats subjected to oral lorazepam (2 mg/kg) and fluoxetine (1mg/kg) administration. Neurochemical analysis suggests that fluoxetine enhances serotonin levels, which counteracts the dopamine-driven addictive effects of lorazepam within the caudate and nucleus accumbens. This supports the notion that serotonin-dopamine interplay facilitates mitigate dependency by stabilizing the reward pathways following lorazepam administration.

Keywords: Addiction, lorazepam, fluoxetine, conditioned place preference, SSRI.

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INTRODUCTION

The neurotransmitter 5-HT, also known as serotonin, intricately regulates a myriad of brain functions and behaviors, exerting control on various functional behaviors (Mitroshina *et al*., 2023). Notably, the potential of serotonergic ligands in treating cocaine abuse disorder, particularly when coupled with comorbid depression, has been highlighted (Jastrzębska *et al*., 2023). A comprehensive body of research underscores the pivotal role played by serotonin-releasing agents and serotonergic receptors in diminishing the abuse potential associated with various substances. Furthermore, impulsivity also involves 5-HT receptors, which is recognized as a central feature contributing to susceptibility to dependence and recurrence, has been extensively explored (Mayer *et al*., 2023; Ranade, 2021). This expanding knowledge underscores the multifaceted impact of 5-HT across diverse neurobiological processes, offering insights into potential avenues for therapeutic interventions in addiction and related disorders. Lorazepam is in clinical practice for the alleviation of pain and anxiety (Safer *et al*., 2023) despite its addictive properties (McCullough *et al*., 2024). Prolonged and indiscriminate use of lorazepam heightens the risk of addiction. At the dose of 2mg/kg, lorazepam demonstrates optimal therapeutic effects with minimal side effects (Ikram *et al*., 2021).

In addition to dopamine, addictive drugs substantially impact extracellular 5-HT activity and 5-HT tissue levels, forming the basis for neurochemical mechanisms that alter behavioral effects and contribute to addiction. Studies indicate important role of serotonin 2C receptors in the addiction (Chao *et al*., 2023). Current study monitored fluoxetine coadministration profile in lorazepam-induced addiction paradigm. A dosage of 1.0 mg/kg fluoxetine was administered, known to increase the release of serotonin in the synapse, thereby enhancing its functional availability (Ma *et al*., 2021). In the caudate and nucleus accumbens, serotonin and dopamine, along with their metabolites, regulate mood and reward pathway (Lewis *et al*., 2021). The hypothesis underlying this study posited that the addictive and reinforcing effects induced by lorazepam could potentially be regulated through an augmented release of 5-HT facilitated by fluoxetine. This exploration delves into the intricate interplay between neurotransmitter systems and addictive behaviors, offering insights into potential pharmacological interventions for managing addiction.

MATERIALS AND METHODS

**Corresponding authors:* e-mails: huma_biochemist@yahoo.com

Animals

The experimental design strictly adhered to the guidelines set forth by the Institutional Animal Ethics Committee (IAEC). Locally bred male Albino Wistar rats weighing between 180-200g, sourced from the HEJ Research Institute of Chemistry, Karachi, were individually housed under 12-hour light-dark cycles and maintained at a controlled room temperature of 22±2ºC. They were provided with free access to tap water and standard rodent diet cubes for 7 days prior to the commencement of the experiment to acclimate to their surroundings. Before initiating the study, the rats underwent familiarization with various handling procedures to minimize environmental stress. All necessary precautions were taken to minimize pain or discomfort. All experimental protocols were approved and conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) and the IAEC (Approval No. IBC-KU-433/2024).

Drugs and doses

Lorazepam as well as fluoxetine (Sigma; St Louis) were dissolved in saline and administered orally at the doses of 2mg/kg and 1mg/kg respectively. Control animals were orally administered with water in volumes of 1.0 ml/kg body weight.

Experimental protocol

Male Albino Wistar rats (24) were divided in a balanced design into four sets of six rats each: (i) water-water (WW), (ii) water-fluoxetine (WF), (iii) lorazepam-water (LW), and (iv) lorazepam-fluoxetine (LF). On day 0, the rats' growth rates had been monitored and meals biscuits have been supplied in their cages. Baseline values were recorded for conditioned place preference test, forced swim test, light-dark box activity, Skinner's box and open field. Oral administration of water (1ml/kg), fluoxetine (1 mg/kg) and lorazepam (2mg/kg) were made to relevant rats. compartments of conditioned place preference apparatus were designated as lorazepam-paired and water-paired relevantly. Rats were treated with lorazepam on alternate days and sequestered in relevant compartments. Behavioral activities were monitored post first and last lorazepam administration. On day 13, place preference in conditioned place preference apparatus was monitored. Rats were then sacrificed, brain regions isolated and stored at -70ºC until neurochemical analysis by HPLC-EC.

Behavioral assessments

Open field activity

Activities were monitored in a novel area of 76×76 cm with 42cm high walls. The procedure was same as described earlier (Ikram *et al*., 2021). Squares crossed with all four paws were recorded for a period of 5min.

Skinner's box activity

Activities were monitored in a familiar area of 26×26×26 cm. The procedure was same as described earlier (Ikram et al., 2024). Cage crossings were recorded for a period of 10min.

Light dark box activity

Two connected compartments, both having equal dimensions of 26x26x26 cm, but differing in their sensory properties. One was light (transparent walls) and the other was dark (black walls). Entries and time spent in compartments were observed over a 5min period, as described earlier (Ikram *et al*., 2024).

Forced Swim Test

Rats were introduced into glass cylinders individually (25 cm high and 10 cm diameter) filled with water at the height of 10cm at room temperature. The animals were tested for a cut off time of 6 minutes (360 seconds). Methodology was same as outlined earlier (Ikram *et al*., 2021; Ikram *et al*., 2024).

Conditioned place preference

Phase I: Pre-conditioning Place Preference Test

The test was conducted as described earlier (Ikram *et al*., 2024). Each compartment was of equal length (26x26x26cm) with a shuttle compartment (12x12cm) between them. The compartments differed in their sensory properties. Walls of one compartment had horizontal grids, while other had vertical grids. Basal values for all rats were monitored with entrances among compartments open.

Phase II: Conditioning Phase

During conditioning entrance between compartments was closed and rats were sequestered in respective compartments for 20min each after drug/saline administration.

Phase III: Post-Conditioning Place Preference Test

On test day (day 13) entrance between compartments was open. Number of entries and time spent in compartments were monitored.

Dissection of rat brain

The dissection procedure was same as earlier (Ikram *et al*., 2024). Rats were decapitated and following removal of skull plates, brain's membranes were delicately detached using fine forceps. The brain, extracted using a spatula, was then rinsed with ice-cold saline. Rat brain slicer was employed to collect samples of caudate and nucleus accumbens and stored at -70°C until analysis.

Neurochemical analysis by HPLC-EC

The extraction process utilized perchlorate (70%), with the addition of a volume five times that of the brain tissues. Electrical homogenization was employed to homogenize the samples, followed by ultracentrifugation (6000 rpm; 20 minutes) at 4ºC. The resulting supernatant

Table 1: Effects of lorazepam, fluoxetine and their coadministration as assessed by three-way ANOVA.

Conditioned place preference		
Treatments	Day 0	Day 13
Lorazepam	F= 0.31; p= 0.25	F= 89.02; p= 0.001
Fluoxetine	F= 0.58; p= 0.56	F= 85.32; p= 0.001
Compartments	F= 0.86; p= 0.27	F= 79.12; p= 0.001
Lorazepam x Fluoxetine	F= 0.52; p= 0.13	F= 51.39; p= 0.001
Lorazepam x Compartments	F= 0.65; p= 0.15	F= 72.16; p= 0.001
Compartments x Fluoxetine	F= 0.94; p= 0.62	F= 81.32; p= 0.001
Lorazepam x Compartments x Fluoxetine	F= 0.61; p= 0.12	F= 64.32; p= 0.001
Motor activities		
Treatments	Novel environment	Familiar environment
Lorazepam (df= 1,80)	F=21.67; p=0.001	F=63.26; p=0.001
Fluoxetine (df= 1,80)	F=29.36; p=0.001	F=73.85; p=0.001
Days (df= 3,80)	F=42.07; p=0.001	F=41.26; p=0.001
Lorazepam x Fluoxetine (df= 1,80)	F=94.23; p=0.001	F=45.76; p=0.001
Lorazepam x Days (df= 3,80)	F=31.47; p=0.001	F=46.32; p=0.001
Fluoxetine x Days (df= 3,80)	F=54.89; p=0.001	F=52.36; p=0.001
Lorazepam x Fluoxetine x Days (df= 3,80)	F=51.10; p=0.001	F=45.40; p=0.001
Anxiolytic and antidepressant profile		
Treatments	Forced swim test	Light dark box test
Lorazepam (df= 1,80)	F=29.89; p=0.001	F=64.21; p=0.001
Fluoxetine (df= 1,80)	F=38.14; p=0.001	F=52.36; p=0.001
Days (df= 3,80)	F=52.03; p=0.001	F=36.81; p=0.001
Lorazepam x Fluoxetine (df= 1,80)	F=48.32; p=0.001	F=51.36; p=0.001
Lorazepam x Days (df= 3,80)	F=34.05; p=0.001	F=53.93; p=0.001
Fluoxetine x Days (df= 3,80)	F=28.73; p=0.001	F=25.36; p=0.001
Lorazepam x Fluoxetine x Days (df= 3,80)	F=26.95; p=0.001	F=23.65; p=0.001

Table 2: Effects of lorazepam, fluoxetine and their coadministration on biogenic amines and metabolites as assessed by two-way ANOVA (df= 1,20; p= 0.001).

Biogenic amines and metabolites in caudate					
Treatments	DA	DOPAC	HVA	5HT	5HIAA
Lorazepam	F= 23.65	F= 39.23	F= 65.21	F= 56.94	F= 74.25
Fluoxetine	F= 64.31	F= 92.64	F= 48.21	F= 91.65	F= 85.32
Lorazepam x Fluoxetine	F= 91.67	F= 87.64	F= 74.53	F= 62.15	F= 64.25
Biogenic amines and metabolites in nucleus accumbens					
Treatments	DA	DOPAC	HVA	5HT	5HIAA
Lorazepam	F= 64.25	F= 89.25	F= 36.54	F= 78.96	F= 76.32
Fluoxetine	F= 68.14	F= 65.27	F= 75.34	F= 65.42	F= 95.76
Lorazepam x Fluoxetine	F= 58.14	F= 71.36	F= 62.01	F= 78.96	F= 62.78

was isolated and utilized for neurochemical evaluation. A Shimpack ODS separation column (ODS-18) was used, with phosphate buffer (0.1 M; pH 2.9) as the mobile phase containing 0.0035% EDTA, 14% methanol and 0.023% octyl sodium sulfate. High (2000-3000 psi) pressure was maintained by HPLC pump (Schimadzu). EC recognition, facilitated by a LEC 6A Schimadzu detector, was achieved with +0.8V operating potential (Ikram *et al*., 2024).

STATISTICAL ANALYSIS

Values are expressed as means ± standard deviation (SD). Statistical analyses involved evaluation of the variances (ANOVA) conducted with software SPSS version 17. Subsequent comparisons between individual groups were conducted using Tukey's evaluation value of $p<0.01$ was considered significant.

RESULTS

A summary of data analyzed by ANOVA, is provided in table 1 (conditioned place preference test, activities in novel as well as familiar environment, forced swim test and light dark box activity) and table 2 (levels of biogenic amines and metabolites in caudate).

DISCUSSION

In this study, rats administered with lorazepam exhibited heightened entries and elevated time spent in the lorazepam-paired compartment. Conversely, rats treated with lorazepam-fluoxetine demonstrated decreased entries and time spent in the lorazepam-associated compartment, suggesting a mitigating effect of fluoxetine on the reinforcing properties of lorazepam. Other research reports have indicated that anxiolytic drugs like

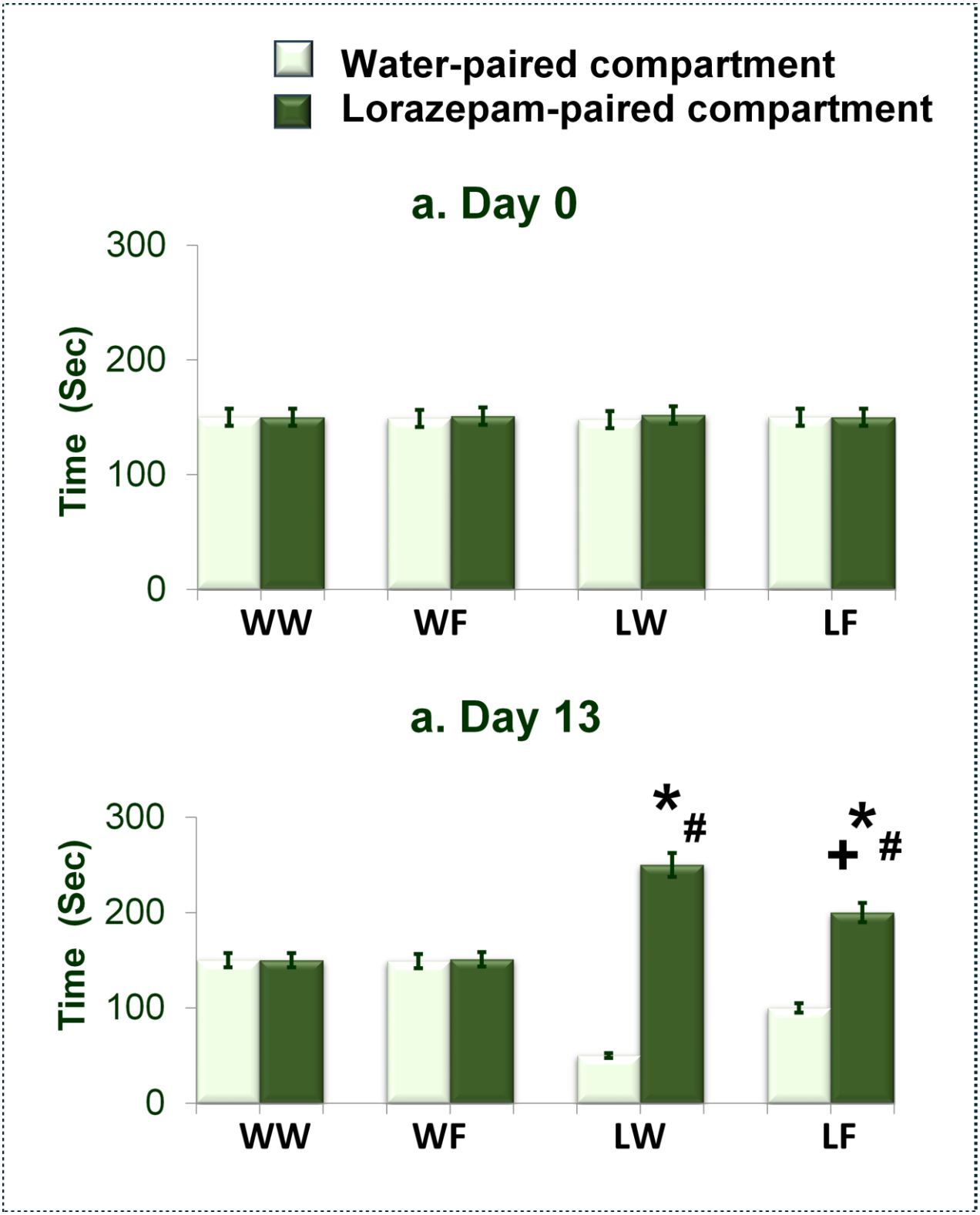

Fig. 1: Effects of lorazepam, fluoxetine and their coadministration on conditioned place preference test. Values are means ± SD (n= 6). Significant differences by Tukey's test: *p<0.01 as compared to respective water-paired compartment; +p<0.01 as compared to respective second water treated group; #p<0.01 as compared to respective first water treated group following 3-way ANOVA.

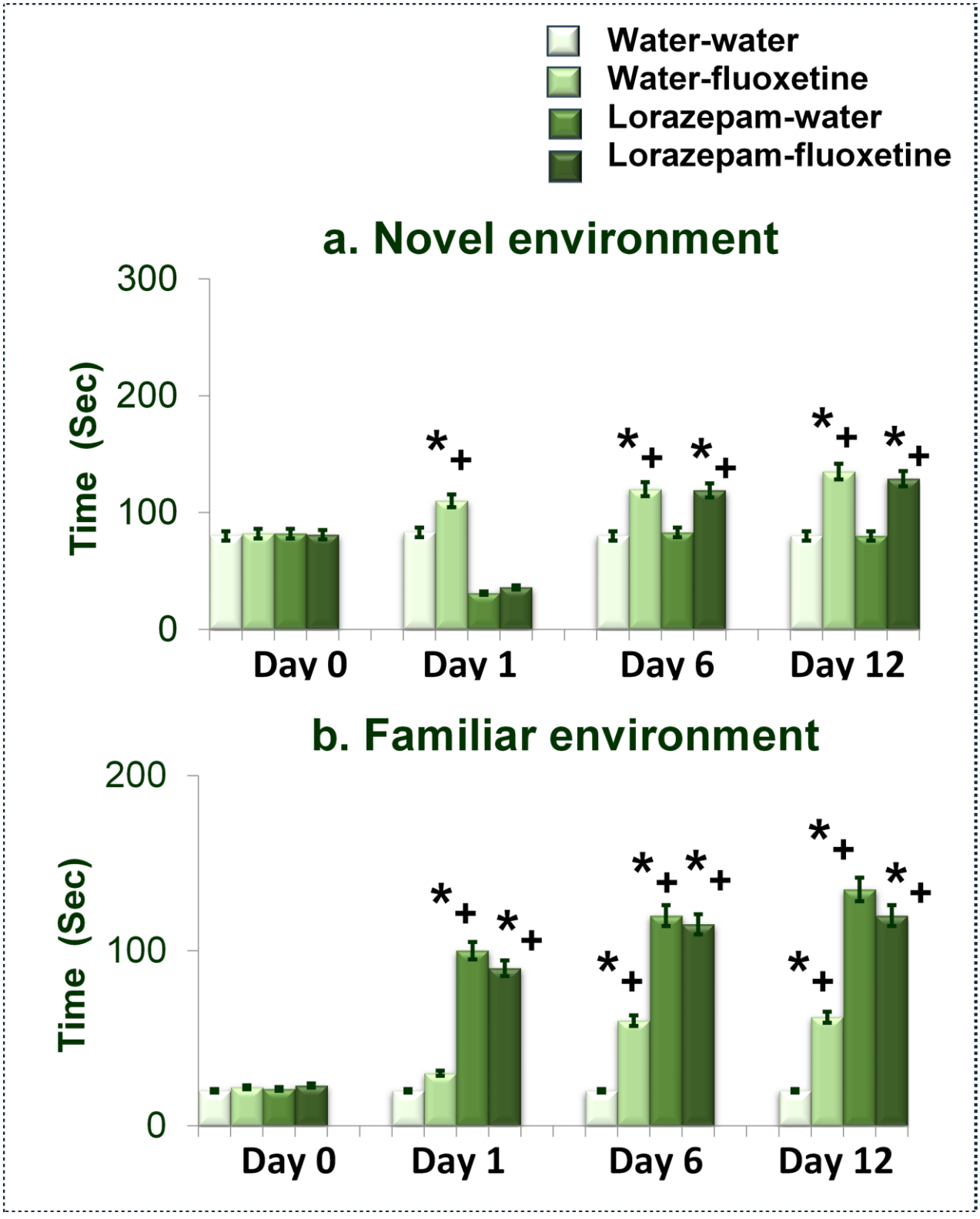

Fig. 2: Effects of lorazepam, fluoxetine and their coadministration on motor activities in novel and familiar environment. Values means ± SD (n= 6). Significant differences by Tukey's test: *p<0.01 as compared to respective water-water group; +p<0.01 as compared to respective day 0 values preceding three way ANOVA.

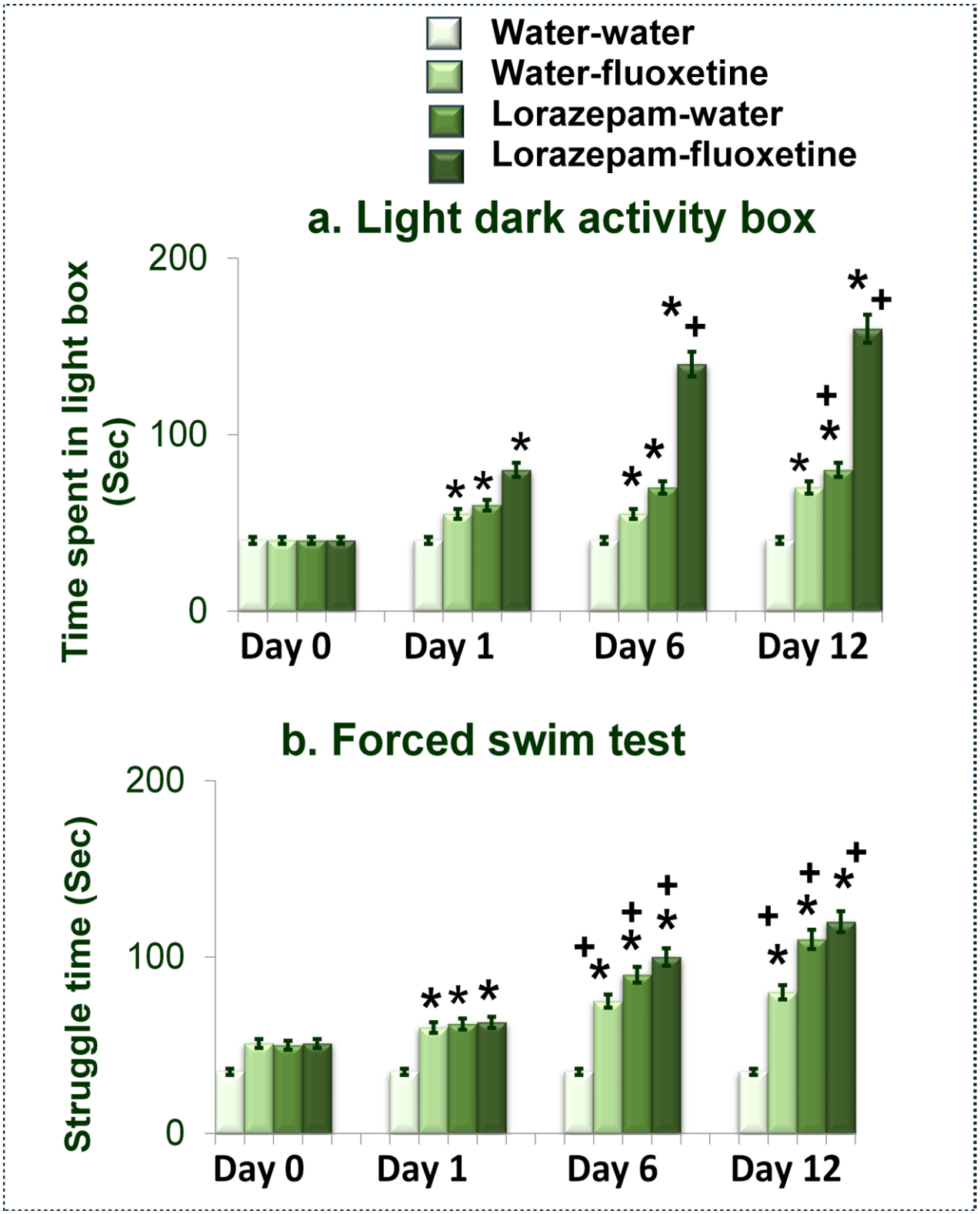

Fig. 3: Effects of lorazepam, fluoxetine and their coadministration on: a. light dark box (time spent in light box) and b. forced swim test (struggle time). Values are means ± SD (n= 6). Significant differences by Tukey's test: *p<0.01 as compared to respective water-water group; +p<0.01 as compared to respective day 0 values preceding three way ANOVA.

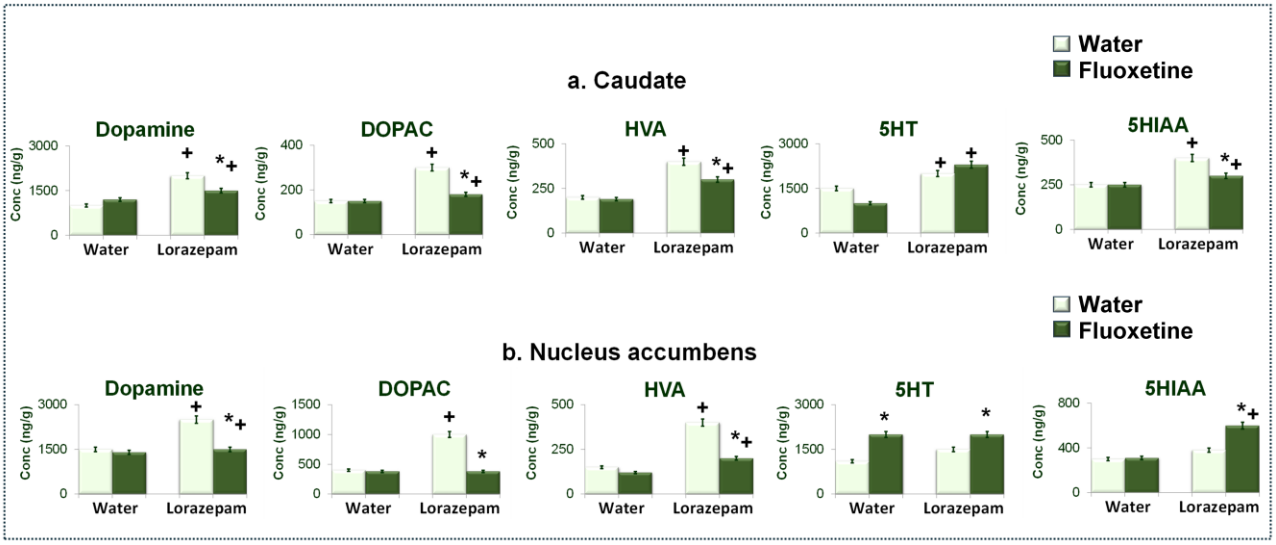

Fig. 4: Effects of lorazepam, fluoxetine and their coadministration on biogenic amines and metabolites in caudate and nucleus accumbens. Values means ± SD (n= 6). Significant differences by Tukey's test: *p<0.01 as compared to respective second water administered rats; +p<0.01 as compared to respective first water treated rats following two way ANOVA.

benzodiazepines, including lorazepam, tend to increase the time spent in drug-paired compartments and the number of transitions between areas (Zamboni *et al*., 2024). The co-administration of fluoxetine appears to counteract these reinforcing effects associated with lorazepam.

The results from this experiment also revealed that lorazepam-administered rats exhibited decreased crossing on days 2 and 12, while lorazepam-fluoxetine-treated rats displayed elevated crossing on these days. Previous studies involving pregnant mice exposed to lorazepam demonstrated elevated activity in the offspring at 3 weeks, although this effect diminished by 6 weeks. Fluoxetine, when administered repeatedly, elevated exploratory activity in an open field (Ikram *et al*., 2021). In our study, lorazepam-administered rats displayed heightened motor behavior in a familiar environment, mitigating the decreased open field crossing observed with lorazepam alone. However, it's noteworthy that motor activity is a crucial component across various behavioral paradigms, and benzodiazepines, including lorazepam, typically induce a sedative effect marked by decreased spontaneous motor activity and exploration (Rombolà *et al*., 2024).

Additionally, lorazepam-fluoxetine and water-fluoxetine groups exhibited elevated struggle time on day 12. Prior research has indicated that benzodiazepine agonists reduce immobility time and increase struggling time in forced swim tests, implying an antidepressant-like profile for benzodiazepines (Haq *et al*., 2023). Surprisingly, high doses of fluoxetine, in both acute and chronic treatments, failed to reduce flat body posture time in the forced swimming testing paradigm (FST) in previous studies (Suman *et al*., 2018). In our study, lorazepam-fluoxetine and water-fluoxetine groups demonstrated decreased latency time, elevated entries, and greater duration of time spent in the light compartment on day 12 in light-dark activity box, suggesting an anxiolytic effect. The anxiolytic effects of benzodiazepines are thought to be mediated through BZ2 receptors located in the limbic system. Research suggests that these results contain the participation of GABA-A receptor α2 and α3 subunits (Lewter *et al*., 2024).

In preceding experimental studies, lorazepam, a normally prescribed benzodiazepine anxiolytic, has been shown to inhibit striatal dopamine release by augmenting the GABAergic inhibitory impact on dopamine neurons (Engin, 2023). Analysis of biogenic amines and their metabolites in our test discovered that lorazepam remedy increased the amount of dopamine, DOPAC and HVA in the nucleus accumbens as well as caudate. These results had been mitigated by means of the concurrent administration of fluoxetine. Additionally, these increased levels were counteracted by fluoxetine co-administration. Elevated levels of DOPAC in the nucleus accumbens and caudate suggest an elevated catabolism of dopamine to DOPAC by intraneuronal monoamine oxidase.

Contrary to other selective serotonin reuptake inhibitors (SSRIs), fluoxetine has been exhibiting acutely increased extracellular concentrations of both dopamine and serotonin in the prefrontal cortex, marking it as an atypical SSRI (Edinoff *et al*., 2021). Our study found that lorazepam-fluoxetine treated rats exhibited elevated 5HT and 5HIAA levels in the nucleus accumbens and caudate. In contrast, lorazepam-treated rats exhibited elevated 5HT

and 5HIAA levels in the nucleus accumbens. This suggests that fluoxetine played a role in increasing 5HT levels in certain brain regions.

CONCLUSION

The concurrent administration of fluoxetine exhibits both antidepressant and anxiolytic activities. Lorazepam administration led to an increase in dopamine levels, an effect that was mitigated by the co-administration of fluoxetine. Concurrent administration of fluoxetine over a two-week period at a dosage of 1.0 mg/kg, in conjunction with lorazepam, mitigated lorazepam-induced reinforcement and behavioral sensitization. This observation leads to highlight the significance of somatodendritic 5-HT1A receptors in the improvement of lorazepam-prompted behavioral sensitization and conditioned Place Preference (CPP). The potential involvement of these receptors' super sensitivity in establishing behavioral sensitization and CPP is suggested, while the repeated administration of fluoxetine leading to desensitization of these receptors may contribute to the attenuation of lorazepam-induced behavioral sensitization and CPP.

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