In Vivo Antidepressant Efficacy of 3-substituted Thietane-1, 1-dioxide Derivative - A Preliminary Study for Novel Anti-depression Therapy in Neurological Disorders

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Research

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Abstract

Background: Psychosocial stress-induced depressive behavior is linked to etiology of several neurological diseases viz., PTSD, and neurodegenerative disease like Alzheimer's disease (AD). The repeated bouts of social stress defeat can be induced using Resident-Intruder-Paradigm (RIP) and chronic mild social stress (CMSS) animal models to assess the stress-induced depressive behavioral patterns. The aim of this study to examine the anti-depressive efficacy of *3-methoxythietane-1,1-dioxide* (N-14) in RIP models of behavioral alterations.

Methods: In this study, we have used Sprague-Dawley rats in Resident-Intruder-Paradigm (RIP), where intruders interacted with residents Day 0 to Day +5 for 10 minutes to invoke CMSS in intruders and became defeated/submissive rats due to the depressive-like behavioral alterations in social activity, explorations, grooming, defense, aggressive behavior, and social interaction, freeze, and rearing etc., with residents. Group I is control intact animals, group II received N-14 alone; group III received CMSS, and group IV received cotreatment of N14 with CMSS. N-14 (2 mg/kg) was administered intraperitoneally from Day 0 to Day +5 to intact animals and intruder animals under conditions of CMSS. Several behavioral tests viz., forced swim test, open field test, and elevated-plus maze test were used to examine the above behavioral dynamic parameters.

Results: the dynamic interaction between Residents and Intruders during the study showed substantial alterations in exploratory activity, aggressiveness, and defensive behavior, body weight, and thymus mass in stressed animals. N-14 cotreatment has mitigated sociability, exploratory activity, and aggressiveness and increased social adaptability and defensive behavior. Extensive rise in active forms of defense and submission latency indicate that N-14 has induced antidepressant activity with a psycho-sedative component of action.

Conclusion: Serendipitously, we observed the ameliorative capability of N-14 cotreatment to mitigate depressive-behavioral symptoms in intruders. Since it is a preliminary study, we have not examined any pathophysiological and molecular signaling to delineate the efficacy of N-14 in retarding depressive-behavioral symptoms. Our future studies will address these aspects to fully consider N-14 as a novel therapy against stress-induced depression in neuropsychiatric and neurodegenerative disorders (ex. AD) using *in vivo* and clinical models.

Background

Alzheimer's disease is associated with dementia constituted by the cognitive decline and functional impairment of neuropsychiatric functions. This disorder currently affecting 5.6 million Americans aged 65 years and the figure is estimated to increase nearly 16 million by 2050. The total payments in 2020 health care and long-term care for AD with comorbid depression patients for aged 65 years and older are estimated to be 305 billion USD [doi.org/10.1002/alz.12068]. The comorbid neuropsychitatirc symptoms (NPS) associated with AD affects daily living, quality of life [1, 2]. In addition, these symptoms are leading to the accelerated disease progression, and mortality [3, 4].

Apathy and depression are the most common comorbid NPS in AD patients. Albeit, several geropsychiatric measures are abundant for diagnosis, the monitoring of depression in AD patients are still remain imperfect [5]. Several pharmacological and nonpharmaclogical interventions are used to treat depression in AD. Depression is the most common NPS in AD patients [5]. Depression induces mild cognitive impairment (MCI) events as studied from meta-analysis of 57 clinical studies; nearly prevalence of 32% in patients with MCI is associated with other depressive symptoms [5–12]. A plethora of studies from clinical setting reported the MCI in AD patients with a rapid, extensive cognitive decline than the non-depressed AD patients [13]. In addition, the beta-amyloid neuritic plaques & neurofibrillary tangles in cerebral parenchyma, serotonin receptor loss, defects in frontal-striatal and frontal-limbic brain pathway are extensively found in AD patients comorbid with MCI than non-depressed AD patients [14–16]. A study reported that 16% of AD patient exhibited depression in a population-based study whereas the 44% of AD patients have attained depression in a hospital-based study; these are indicating depression as an early manifestation of AD [17, 18].

Several risk factors were reported to develop depression during neurological diseases; mainly the risk factors viz., the familial history of depression, female sex, ApoE-4 positivity, and the usage of certain medications [19–21]. For instance, the intake of beta-*adrenergic receptor blockers, corticosteroids, benzodiazepines*, recurrent exposure to *dopamine antagonists, statins, proton-pump inhibitors, anxiolytics, hormone-replacement therapies, anticonvulsants*, and *anticholinergic drugs* like dicyclomine, and prolonged exposure to *CNS stimulants* are likely induces the *depressive disorder* in people exposing to prolonged chronic mild social stress, both depressed AD & non-depressed AD patients, and stroke patients, and [5]. Thus, depression is a critical issue with both medically and socioeconomically implications [5].

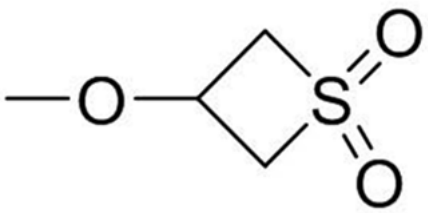
Depression is accompanied by the social risk factors, diminished cooperativeness, and competition avoidance due to behavioral alterations. The chronic social stress like trauma, abuse, frequent illness, fear etc. will likely to enhance the depressive symptoms in patients with neurodegenerative diseases like AD [22]. In addition, the depression is also associated with the '*social anhedonia, hypersensitivity to social rejection, impaired social communication, impaired aggression, and impaired emotion recognition*'. These kind of dysfunctional social processes in *animal models of social stress* are attributed to the behavioral, neuroanatomical, and genetic levels [22].

Antidepressants like *tricyclic antidepressants* such as amitryptiline, imipramine, *selective serotonin reuptake inhibitor*s (SSRIs) such as fluoxetine, paroxetine etc., are preferred to treat major depressive symptoms viz., altered mood *libidos*, stress-induced behavioral alterations in neurodegenerative diseases (ex. AD), postpartum depression, and postschizophrenic depression etc., [23]. The moclobemide is ineffective in relieving symptoms of depression with no further improvement in cognitive function [24, 25]. Fluoxetine, sertaline, citalopram are normally prescribed for AD patients with depression. However, their usage is constrained by the additional comorbid cognitive decline, and nausea, vomiting, diarrhea, anorexia, weight loss, dizziness, constipation, and dry mouth, anxiety, and insomnia etc., Therefore, the selection of a particular antidepressant must encompass the adverse effects to treat comorbid symptoms such as *behavioral alterations* in depression [5, 23]. However, therapeutic effectiveness of antidepressants varies by individual and may have serious behavioral abnormalities and other side-effects. Therefore, there is an urgent need for the development of new and more efficacious antidepressants [5]. Unfortunately, a

large proportion of promising molecules in preclinical studies have been shown to be ineffective in clinical trials stage. The actual consequences for clinical failure of several antidepressant molecules are associated to the lack of assessment of existing models of depressive-like states [5, 26, 27].

In addition, we have examined the safety profile and activity of new promising lead compounds from the *3-substituted thiethan-1,1-dioxides* class (Ex., 3-methoxyethane-1,1-dioxide, *laboratory code N-14*). First time, we have examined the efficacy of heterocyclic compound N-14 and there is no current literature available. N-14 showed antidepressant effects and low toxicity in the *in vivo* model [28, 29]. Alternatively, the models based on "social stress & resident-intruder paradigm" are predictive and can be validated. These model methods are efficiently beneficial to invoke behavioral and homeostatic behaviors in animal models in a semi-natural environment. In addition, these methods significantly beneficial for examining the efficacy of antidepressants to reverse abnormal behaviors associated with depression [27]. We used modified RIP that enable the assessment of aggressive and defensive behaviors [30]. The present research is a '*preliminary study'* to examine the psychopharmacology of "3-substituted thietane-1, 1-dioxide **derivative (N-14)"** *in vivo* models of invoking depression through social stress; this could be a 'prospective novel animal model' to determine the effects of stress on adolescent neurobehavioral phenomenon consequently beneficial to develop novel nontoxic therapeutic intervention against comorbid depression using *in vivo* models of neurodegenerative diseases including AD.

Chemical Structure



Structure of N-14 (*3-substituted thietane-1,1-dioxide* derivative)

Methods

Experimental design

Animals: All animal procedures were carried out in accordance with the International Recommendations of the *European convention* for the protection of vertebrate animals used for experimental and other scientific purposes [31]. These procedures were approved by the Ethical Committee for animal care of the Bashkir State Medical University, Ufa, Russian Federation. The experimental subjects were white male Sprague Dawley rats [32]. They were kept in standard vivarium conditions with a 12/12 h light/dark cycle (lights off at 20:00) and provided water and food *ad libitum*.

Before experiment, all male rats were categorized into "Residents" and "Intruders" [32]. Resident male rats weighing 250–350 g were housed in large individual cages (90*60*50 cm) for more than 2 weeks to facilitate the development of territoriality. Each male was provided a sterilized companion female one week before the test to enhance territoriality and prevent effects of social isolation. Two days before the test, Residents interacted with small non-experimental male rats for 10 minutes twice a day to develop dominance behavior.

Intruders were male rats of smaller size (150–250 g). They were randomly assigned to 4 experimental groups:

Group I

Intact animals (control)

Group II

Animals treated with N-14 on Days from 0 to + 5

Group III

Animals that underwent resident-intruder CMSS on Days 0 to + 5

Group IV

Animals that underwent CMSS and received N-14 on Days from 0 to + 5; on + 6th day, animals were ethically sacrificed

	No N-14	Received N-14
No CMSS	Group I	Group II
Received CMSS	Group III	Group IV

The idea of the experiment is to develop depressive-like symptoms in Intruders due to repeated bouts of social defeat incurred by chronic mild social stress (CMSS) during *Resident-Intruder* interactions and N-14 cotreatment used to reverse these symptoms.

Drug administration

LD-50 of N-14 was determined as per *Ivanova, O.A., Nikitina, I.L et al (2011)* [28]. N-14 was suspended in Tween 80 and dissolved in 0.9% NaCl solution and prepared 2 mg/kg concentration. Rats were treated daily with N-14 or saline intraperitoneally in a volume of 2 ml/kg on days from 0 to +5.

Procedures

Chronic bouts of social defeat were induced in intruders from chronic mild stress (CMSS) when interacting with residents in the cage [32, 33]. Intruders interacted with Residents for 10 minutes daily for 6 days (i.e., from Days 0 through +5). Interactions occurred during the dark phase. Interactions between residents and intruders were recorded using a Panasonic V760 camera.

The behavior of the Intruders was analyzed by computing the duration of the patterns and types of behaviors using *BrainTest* 2.0 program [34]. Trained observers collected data on social behaviors as follows:

1. Latency of First Interaction
2. Latency of Submission
3. Social Exploration
4. Non-Social Exploration
5. Sitting
6. Social Interaction, operationally defined as the sum of the patterns Fight and Attack
7. Social Inactivity, operationally defined as the sum of the patterns Rearing, Recline Against Wall, and Moving
8. Grooming
9. Defense, operationally defined as the sum of the patterns Move Away, Flight, Submissive Posture, Freeze, and Defensive Upright Posture

On Day +5, Intruders were tested using the Forced Swimming Test (FST) [35]. On Day +5 they were tested in the Open Field (OF) and Elevated-Plus Maze (EPM) tests [36].

Forced Swimming Test (FST)

A subset of intruder Sprague Dawley rats experiencing CMSS underwent FST to ascertain the depressive-like behavior; and later, to assess the N-14 efficacy to ameliorate the behavioral alterations [32]. FST is composed of two testing days (on Day +5, Day +6), only the time spent immobile on 2nd day could be inferred as the behavioral indicator of despair whereby the expressing urge to escape from adverse stimuli diminished. Initially the rats after subjecting to CMSS were placed in the plastic cylinder [r ¼ 30.5 cm; h ¼ 46.0 cm] composed of three liters of water and maintained them at room temperature for 15 minutes. Subsequently, the procedure was repeated again next day for 5 minutes. Here, immobility is referred as the time spent motionless on the water surface without any submerged limb movement in the water was recorded for every rat using Zeiss (ZSD-808) Stopwatches. Higher immobility was considered as the depressive-behavior, which can be ameliorated by the administration of anti-depressants [37]; Mean ± SEM immobile time during Day 2 (in 5 min) was noted for all the intruders.

Open Field (OF)

Open field activity of intruders experiencing CMSS was recorded using Opto-Varimex-3 Activity Meter (Columbus Instruments, Columbus, OH, USA) coupled with a standard, open, Plexiglas arena. The animal movement was recorded using infrared sensors by placing them 3 cm above the floor as described by *Wei S et. al., (2014)* [33]. Each rat was placed in the centers of apparatus prior to the testing consequently allowed it to explore for at least 3 minutes. At this time, the open-field activity was recorded and the other exploratory dynamics viz., rearing etc., were observed with care and quantified these parameters [33].

Elevated Plus Maze (EPM) test

EMP is composed of plus-shaped platform situated above 80 cm from the floor. It consists of two open arms (50×10 cm; 100 lux) and two closed arms (50×10×40 cm; 20 lux). Intruder rats were placed across the center square towards the closed arm. The following parameters were recorded for 5 minutes EPM test using *Plus-maze version 2.0*, [38], *Ernst Fricke* software; For instance, the parameters are,

—> time spent in open and closed arms

—> number of entries into open and closed arms

—> latency to enter an open arm

Additionally, the **weight gain** of and the amount of **food consumed** by Intruders (comparing Days -1 to +5) were measured and coded in terms of 100 g of animal weight. At the end of the experiment, the animals were euthanized ethically. To assess the anti-depressant effect of N-14 against stress-induced behavioral alterations, we have analyzed several behavioral patterns like social inactivity, exploration, grooming, defensive behavior, and aggressive behavior. In addition, we have examined the masses of the liver, spleen, thymus, and adrenal glands were measured.

Statistical Analysis

Statistica 10.0 (StatSoft, USA) was used for data analysis. Descriptive statistics included the median (M) and interquartile range (IQR). Mann-Whitney test was used for pair-wise comparison of variables; Wilcoxon test was used for dependent samples analysis [39]. For all statistical analyses, the significance level was p=0.05.

Results

Resident-Intruder interaction

N-14 cotreatment induced behavioral alterations related to Social Interaction, Social Exploration etc.,

Social Interaction is associated with inclusive actions of Fight and Attack. N-14 cotreatment in Group IV significantly mitigated Social Interaction on Day + 2 ($p < 0.05$) when compared to Group III animals. The *Attack pattern* was reduced 4.1–15.8 times on all days of the experiment, with statistical significance on Day 0 compared to Group III.

When compared to Day 0, the level of *Social Interaction* was decreased due to decline in the *Attack pattern* with and without N-14. However, this decline was statistically insignificant. The level of aggressiveness was declined in Group IV received N-14 cotreatment compared to Group III (CMSS only). This kind of behavioral alteration in the Social Interaction of Intruders under CMSS indicates alleviation of aggressiveness.

Social Inactivity can also be determined through the behavioral alterations pertaining to the sum of *Rearing, Recline Against Wall*, and *Moving*. *Social Inactivity* was decreased with N-14 cotreatment in Groups IV on Days + 1, +2, + 3 ($p < 0.05$) compared to Group III; further *social inactivity* was associated with a reduction in the total duration of *patterns Moving* on Days + 1, +2, + 3 ($p < 0.05$), *Recline Against Wall* on Day + 2 ($p < 0.05$), and *Rearing* on Days + 2 and + 3 ($p < 0.05$). However, N-14 cotreatment reduced *Social Inactivity* on Days + 3 and + 5 also due to decrease in the total duration of *patterns Moving* and *Recline against Wall* compared with Day 0 (statistically insignificant). In Group III, *Social Inactivity* was increased on Days + 2 and + 3 but decreased on Day + 5 (statistically insignificant). Such dynamics are due to changes in *Moving* (164% on Day + 2 and 189% on Day + 3, $p < 0.05$) and *Rearing* (39% on Day + 5, $p = 0.173$) when compared to Day 0. Finally, it can be deduced that the change in *Social Inactivity* indicates a decrease in *exploratory activity* of animals with N-14 cotreatment. Our results significantly correlated with N-14-induced mitigation in the *Non-Social Exploration* ($p < 0.05$) on Day + 5, which goes along with Social Inactivity.

Grooming

Grooming pattern was significantly modulated in Groups III and IV. This effect was evident on all experimental days in Group III where maximum values were observed on Days + 1 and + 4, $p = 0.08$ and $p = 0.075$ respectively. This total duration of pattern was declined significantly with N-14 cotreatment in Group IV on Days + 1, +4, and + 5 when compared to Group III ($p < 0.05$ on Day + 3).

Social Exploration

N-14 cotreatment also reduced *Social Exploration* pattern on all days of the experiment when compared to Group III ($p < 0.05$ on Days 0 and + 3).

Latency of First Interaction

Latency of First Interaction was increased in Groups III and IV when compared to Day 0 ($p < 0.05$ on Day + 1, Group III); N-14 cotreatment enhanced the pattern value in Group IV on Days + 1 (161%), + 4 (340%) and + 5 (225%) when compared to Group III.

Defense

Defensive behavior was higher in the group of stressed animals cotreated with N-14 ($p < 0.05$, only on Day + 3) than in Group III due to the *Freeze pattern*. Freeze was higher on all experimental days ($p < 0.05$, only on Day + 3) compared to the stressed animals of Group III (the proportion was 87–94%, **Figure-3**). Unidirectional patterns of Defense and Freeze behaviors were observed in N-14 cotreatment in Group IV; there was a general decrease in Defense and Freeze behaviors when compared with Day 0 on Days + 2, +4, + 5. The Defense behavior on Day + 1 was affected by a significant decrease in *Defensive Upright Posture* with N-14 cotreatment than Day 0.

In case of Group III stressed animals, there was a tendency to decrease in *defensive behavior* on all days; which might be due to reduction in the *Submission Posture* on Days + 1, +2, + 3, +5 (statistically insignificant) and *Freeze* on Day + 4.

During the experiment, passive forms of defense viz., *Freeze* and *Submissive Posture* prevailed in the behavior structure of animals of both groups. This was caused by individual behavioral alterations of the Intruders rather than specific response to CMSS [40]. The largest proportion of *passive defense* in animals treated with N-14 was *Freeze* (87–94%, **Figure-3**); and it was declined on Days + 4 and + 5 compared to the other days. In case of Group III, the passive defense was represented by both *Freeze* and *Submissive Posture* on Days + 1 to + 4, with an extensive propensity for the use of *Submissive Posture*.

N-14 cotreatment in Group IV induced a significant decrease in *Move Away pattern* on Days 0, + 2 and + 3 and *defensive behavior* of Intruders (1–2%, in stressed animals − 9–15%) compared to the Stress Group III. In addition, N-14 significantly reduced the *Defensive Upright Posture* on Days + 1 ($p < 0.05$), + 4 and + 5 when compared to Group III, which correlates with alleviation of *aggressive behavior* in N-14 cotreatment and reflects the development of "adaptive survival strategy".

At the same time, N-14 cotreatment promoted extensive increase in the *patterns of Defensive Upright Posture* on Days + 2 and + 3 as compared to Day 0 (the reverse trend was observed in the control group); N-14 also fostered the rise in *Flight* on Days from 0 to + 3 compared with both Day 0 and Group III. This is indicating the N-14 induced manifestation of the antidepressant effect.

Latency of Submission

The antidepressant activity of N-14 was confirmed by an increase in the *Latency of Submission* when compared to Group III (1.5–10 times on Days + 2 to + 5), and in the dynamics relative to Day 0 (1.4-9 times on Days + 1, +3, + 4, +5, $p < 0.05$), with the behavioral pattern reaching its maximum on Day + 5 (Me = 488.3 s).

The results of Resident-Intruder interaction are shown in **Table-1**. Additionally, we calculated the proportions of each pattern in the structure of Intruders behavior on Days 0 to + 5. They are given in **Fig. 2–3**.

Pattern / *Type of behavior*	Day 0		Day +1		Day +2		Day +3		Day +4
	Stress (III)	Stress + N-14 (IV)	Stress (III)	Stress + N-14 (IV)	Stress (III)	Stress + N-14 (IV)	Stress (III)	Stress + N-14 (IV)	Stress (III)
Attack	15,8 [4,5;33,5]	0,0* [0,0;0,3]	7,3 [0,0;21,3]	0,0 [0,0;0,8]	5,1 [0,5;45,0]	0,0 [0,0;0,2]	4,1 [0,0;22,2]	0,0 [0,0;3,2]	11,6 [4,0;40,3]
Fight	4,2 [2,8;8,3]	6,5 [3,6;16,2]	3,7 [0,8;6,1]	2,0 [0,0;3,2]	4,7 [2,3;6,9]	4,1 [1,8;6,2]	2,0** [0,9;5,5]	5,8 [2,8;6,4]	1,8 [1,2;4,5]
Social interaction	26,4 [8,2;50,8]	6,7 [3,6;16,4]	10,5 [1,7;27,1]	2,1 [0,0;5,3]	14,3 [7,8;47,3]	4,2* [1,8;6,2]	10,9 [1,8;24,4]	5,9 [5,8;14,8]	12,5 [6,0;41,9]
Rearing	9,6 [6,0;13,9]	8,9 [2,8;14,3]	5,6** [3,6;8,0]	0,0 [0,0;1,8]	7,7 [5,9;13,5]	3,6* [0,0;4,8]	6,6 [5,4;10,6]	3,6* [0,2;4,9]	2,5** [2,0;9,1]
Recline against wall	17,1 [2,2;22,1]	11,6 [2,7;17,5]	7,6 [3,7;15,9]	3,7 [0,9;5,2]	15,3 [12,7;21,1]	2,3* [0,0;10,9]	16,2 [12,1;21,9]	5,1 [1,0;6,5]	6,4 [1,0;16,1]
Moving	44,5 [22,9;73,4]	8,3 [4,5;19,6]	51,4 [37,0;77,0]	19,9* [9,7;25,4]	73,1** [58,2;92,2]	7,4* [0,0;19,7]	84,1 [55,0;100,1]	3,6* [0,0;6,9]	42,4 [22,7;87,9]
Social inactivity	71,2 [31,1;109,4]	28,4 [19,8;87,9]	60,3 [50,3;106,7]	28,5* [12,0;31,9]	97,8 [78,8;138,2]	23,1* [0,0;41,1]	106,5 [95,9;129,7]	10,0* [6,8;19,9]	56,4 [26,2;106,3]
Grooming	3,4 [0,7;9,4]	0,0 [0,0;0,0]	38,0 [9,9;66,6]	4,0 [0,0;14,0]	23,6 [12,2;45,2]	0,0 [0,0;14,9]	20,9 [5,3;42,6]	0,0* [0,0;0,5]	45,5 [6,7;101,2]
Non-social exploration	80,9 [70,0;158,2]	53,8 [35,2;61,9]	87,2 [70,3;155,0]	55,9 [41,9;164,5]	133,4 [40,3;177,8]	52,9 [1,6;116,7]	148,1 [94,8;179,7]	50,6 [4,6;104,1]	128,5 [65,3;140,6]
Social exploration	62,3 [38,3;128,4]	12,6* [6,8;24,0]	80,6 [44,1;104,8]	36,7** [34,0;66,5]	58,1 [53,8;90,8]	4,9 [0,0;78,8]	68,8 [65,6;88,1]	0,3* [0,0;1,2]	69,3 [46,8;96,9]
Move away	22,4 [12,7;33,5]	4,9* [3,8;9,9]	13,2 [6,5;19,4]	4,4 [0,8;10,1]	21,9 [15,8;30,1]	1,4* [0,9;4,3]	17,6 [11,3;23,0]	2,6* [1,2;10,9]	10,7 [8,3;13,6]
Flight	0,0 [0,0;0,0]	0,3 [0,0;6,2]	0,0 [0,0;0,0]	0,4 [0,0;1,8]	0,0 [0,0;0,0]	0,6 [0,0;8,3]	0,0 [0,0;0,0]	0,6 [0,0;2,9]	0,0 [0,0;3,0]
Submissive posture	62,1 [31,6;84,0]	33,1 [26,2;50,9]	13,2 [3,0;55,4]	14,0 [0,0;27,1]	17,0 [13,7;38,5]	27,1 [6,4;54,1]	43,7 [29,0;91,0]	24,8 [0,3;84,5]	63,2 [1,6;97,5]
Defensive upright posture	14,0 [4,2;33,3]	4,3 [2,7;10,5]	9,7 [1,2;23,2]	0,6** [0,0;2,7]	12,2 [6,9;17,3]	6,1 [0,9;13,3]	6,8 [2,7;9,9]	5,4 [2,2;17,4]	11,4 [0,7;34,1]
Freeze	97,3 [65,1;228,7]	360,8 [353,6;443,3]	108,3 [70,4;277,5]	310,3 [237,0;353,0]	93,1 [54,2;120,1]	240,0 [159,0;371,7]	76,8 [17,7;228,1]	265,8* [227,7;446,7]	40,2 [33,8;204,4]
Defense	293,4 [156,5;364,7]	460,9 [402,7;501,6]	189,6 [131,5;283,8]	317,9 [287,8;380,0]	192,5 [115,6;202,9]	267,7 [238,4;418,0]	185,3 [125,2;293,3]	409,8* [285,8;479,2]	149,4 [118,9;223,4]
Latency of first interaction	2,3 [2,0;2,4]	1,7 [1,3;11,3]	3,4** [2,8;7,1]	5,5 [3,0;10,6]	3,5 [3,2;4,5]	3,4 [1,8;7,7]	5,2 [2,1;7,3]	3,6 [2,9;7,2]	4,2 [2,0;5,0]
Latency of submission	50,2 [30,5;76,7]	56,8 [11,6;90,3]	67,0 [58,4;76,6]	77,7 [63,3;600,0]	31,7 [17,3;35,2]	45,6 [25,8;264,7]	38,2 [35,8;61,6]	156,0 [17,8;558,7]	64,8 [14,7;133,8]
Sitting	22,9 [6,1;33,4]	11,8 [5,3;49,8]	37,1 [23,6;44,2]	65,5 [3,4;163,2]	5,7 [1,3;34,0]	6,0 [0,0;318,2]	0,0 [0,0;17,1]	0,1 [0,0;101,0]	17,7 [0,2;22,6]

Table 1

Table-1: Effect of N-14 and Social Stress on the behavior of Intruder animals in the Resident-Intruder Paradigm

Notes:
1) Table shows median (Me) and interquartile range ([25%; 75%]).
2) The asterisk (*) indicates statistical significance (p<0.05) for the Mann-Whitney test relative to Group III.
3) The double asterisk (**) indicates statistical significance (p<0.05) for the Wilcoxon test relative to Day 0.

N-14 cotreatment induced alterations in 'duration of immobilization' (DIM-Forced swimming test)

In the FST [10], there was an insignificant decrease in the duration of immobilization (DIM FST) of Group III stressed animals compared to the Group I control. N-14 did not affect DIM FST in both stressed (statistically significant) and intact animals.

N-14 cotreatment induced alterations in 'Exploratory Activity, Sitting, Hole, Rearing, Sitting, and Moving' (Open field test)

N-14 cotreatment invoked a significant decrease in Exploratory Activity (26%), Moving (67%), and Sitting (40%) in stressed animals on Day + 5 when compared to the Group III. In Group II, N-14 caused a slight increase in Exploratory activity (129%), Hole (133%), and Moving (200%). There were insignificantly reduced patterns of Rearing (40%) and Sitting (50%) compared to the intact control (Group I).

N-14 cotreatment induced alterations in 'open arms time, center time, open arms entries, and closed arms returns' (Elevated plus-maze test)

There was a slight increase in the *Open Arms time* in Group III by 47% compared to the intact control Group I. There was reduction in *Open* and *Closed Arms entries* by 33% and 25%, respectively; concomitantly, reduction in *Closed Arms Returns* (by 33%) and *Head Dipping* over the sides of the maze (by 150%) was observed when compared to the control Group I. There parameters were significantly altered by the cotreatment of N-14 to CMSS animals. N-14 cotreatment invoked reduction in the *Open Arms time* in the Group IV- stressed animals, near normal to Group I intact animals. The number of *Closed Arms Returns* and *Closed Arms entries* significantly increased with N-14 when compared to Group I Intact (by 167% and 25%) and Group III Stressed (by 100% and 67%). In intact animals, N-14 induced significant reduction in the *center time* and *closed arms entries* with simultaneous increase in the *open arms time*, *open arms entries*, and *closed arms returns*.

Effect of N-14 cotreatment on Weight gain and food consumption

CMSS significantly induced weight gain of the Intruders (Δ_{0-+6} = 28.5 g, p = 0.009) but did not affect the amount of food consumed by Intruders. Moreover, N-14 did not affect the *weight* of intact animals or their *food consumption*; these parameters were reduced by 29 g (p = 0.004) and 4.5 g respectively in the Group IV stressed animals. Group IV individual food consumption was lower than the Group III significantly on Days + 4 and + 5.

Effect of N-14 cotreatment on the mass of internal organs viz., thymus, and liver

Interaction between Residents and Intruders caused profound increase in the mass of thymus (135%) in Group III stressed animals. A similar trend was observed in the group of animals treated with N-14 (Group II). The administration of N-14 to animals that underwent CMSS (Group IV) induced profound decline in the mass of thymus by 55% compared to Group III, (p = 0.065); similarly, the liver mass was also significantly decreased by 13% in Group IV (p = 0.026) compared to Group III.

Discussion

Currently, there are no significant molecular therapies against the depression induced in several neurological diseases, neurodegenerative diseases like AD, and trauma conditions. Short-term "modern-life stress", "chronic isolation stress", "chronic mild/variable stress", "chronic mild social stress" are reported to be increase the pathophysiology of AD and comorbid depressive symptoms with cognitive impairment [41-46]. The present preliminary study examined the anti-depressive efficacy of *3-substituted thietane-1,1-dioxide* using RIP-stress induced models. It was concluded that the adolescent *Sprgue-Dawley* male rats exposed to chronic mild stress exhibit a depressive phenotype eventually exemplified by the hippocampal fear-conditioning, and synaptic plasticity [32, 47, 48]. In order to examine whether the RIP-induced behavioral alterations are accompanied by depression during mild social stress, we have used *Sprague-Dawley* rat models of daily exposure to mild social stress from day +0 to day +5 [33]. *Sheng Wei et al (2014)* delineated that the RIP induced aggressive behavior in the intruders [33]. Reverse-Resident-Intruder-Paradigm (rRIP) is reported to be an efficient model to examine the effects of stress on adolescent neurobehavioral phenomenon [32]. The major findings of our study is that the CMSS has induced 'depressive-like behavior' in male adult intruder rats, which was relieved by the cotreatment of N-14 in the group of animals which underwent chronic mild stress. There was an initial dynamic interaction between residents and intruders during the experiment, which was exemplified by the increase in the exploratory activity through nonsocial exploration.

The social defeat model from chronic social stress summarized the association between behavioral and physiological effects relevant to depression in many neuropsychiatric and neurodegenerative diseases. *Fiona Hollis et al (2014)* have reviewed the suitability of the social defeat to the understanding of psycho-neurobiology of depression and the potential avenues to develop novel therapies against behavioral alterations during depression in neurodegenerative diseases [49]. Normally, the intruders in the RIP exhibit defensive and aggressive behavior in response to offensive attacks by residents [50]. Hence, this paradigm is significant model to ascertain the behavioral aspects like defense in relation to social stress using intruder as the major experimental animal. We have not introduced social stress in the same room where the non-stressed controls were housed because control animals may experience major stress when they witnessing social stress [51, 52]. However, the intruder may face dire consequences during defense against residents and experience severe depression, anorexia, loss of body weight due to social defeat from chronic social stress [30]. Our study vividly reported a significant decrease in body weight and increase in the weight of thymus of intruders due to the recurrent depressive symptoms due to social defeat from residents. N-14 cotreatment did not affect either weight or amount of food consumption in the intruders but mitigated the mass of thymus.

The forced-swimming test is predominantly suitable to determine the anti-depressant activity of novel lead molecules [53-57]. The reduced immobility time in this test was indicated for anti-depressive efficacy that countered the behavioral expression of depressive-symptoms in rats [58]. Previous reports from *Koolhaas J et al (1990), Bielajew C et al (2003)* delineated the behavioral alterations from social defeat from chronic mild social stress [58, 59]. In our study, CMSS mitigated the social interaction of intruders both in the dynamics compared to Day 0 and in comparison with Group III. This data indicate a decrease in aggressiveness of animals due to the development of an *Avoiding Conflict strategy* and increasing *social adaptability*. In Group IV, N-14 cotreatment significantly mitigated the level of aggressiveness than in stressed animals of Group III alone. N-14 cotreatment induced the extensive *social interaction* of Group IV animals throughout the experiment compared with Group III stressed animals, although there was a decrease in this type of behavior in Group III on Days +3 & +5 whereas on Days +4 and +5 in group IV.

In the forced swimming test, CMSS invoked reduction in the DIM (***duration of immobilization***) FST. N-14 did not affect DIM in FST of stressed animals significantly, which may be due to test deficiencies: it has been shown that interventions affecting the locomotor activity of animals led to a change in DIM and, accordingly, to false positive or negative findings [60]. Therefore, in order to evaluate the antidepressant effect of the compounds with a sedative component, it is advisable to use a *depression index* i.e. the ratio of the number of short [less than 6 s] immobilization periods to the number of active swimming periods [29].

Social Inactivity was decreased in group IV rats due to extensive decline in the *exploratory activity* N-14 cotreatment. This finding may be due to the psycho-sedative effect of N-14. The Non-Social Exploration pattern also reflects the level of exploratory activity, which was changed unidirectional with Social Inactivity. N-14 significantly alleviated its total duration than stressed animals. This kind of behavioral patterns with N-14 cotreatment vividly delineated its ability to mitigate the development of behavioral despair similar to other anti-depressants [56, 61, 62]. In our study, the cotreatment of N14 with animals underwent social stress have resulted in the higher motivation and impaired behavioral despair.

Initial activity of the rats located in any new environment (for ex. an open field) can be considered as an indicator of its emotion and motivation state [63-65]. Inescapable open filed often trigger stress and reward behavior of novelty consequently induces impaired locomotor and exploratory activity in new environment [66, 67]. However, the reduced exploration of a novel environment may be related to higher anxiety levels in stressed animals. We have not examined whether the observed changes in locomotor activity were due to altered anxiety level [33]. Social Inactivity and Non-Social Exploration are indicators of the social passivity of animals along with the Grooming pattern; The grooming pattern was also decreased under the cotreatment of N-14 in stressed animals. N-14 significantly reduced the sociability of animals, which was evident by its activity at the patterns of *social exploration* and *latency of first interaction*. This may indicate an increase in the sociability of animals although there was substantial rise of *latency of first interaction*.

A report by *Sheng Wei et al (2017)* described the ability of residents to defend intruders due to their emotional aggressiveness; the administration of fluoxetine to the intruder groups mitigated the aggressive behavior and resident-intruder stress [68]. It was well established that the RIP confer aggressive behavior in male rats; which can be considered as the model of depression to evaluate the aggressive behavior upon anti-depressant therapy [33, 69]. In our study, N-14 significantly increased Defense behaviors throughout the experiment in the both dynamics when compared to Group III. N14 cotreatment enhanced adaptive survival strategy which was evident from the Passive forms of Defense behavior prevailed in Intruders' behavior, where the predominant pattern observed was Freeze behavior (87-94%). In addition, N14 invoked reduction in *Move Away* and *Defensive Upright Posture* compared to the Stress Group, which is correlated to the decline in aggressiveness by N-14. There was an increase in the active forms of Defense behaviors: *Flight* & *Latency of Submission* when compared to Group III. This is apparently a significant evidence for the antidepressant activity of the compound N-14 with psycho-sedative properties. However, the dynamics (Days 0 to +5) showed a tendency for the Defense behaviors to increase: Day +1 (48.6%) and Day +5 (120.7%). This is due to the reduction in the duration of *Freeze* on all days of the experiment and *Submissive Posture* on Days 0, +2, +3 compared to Group III. Previously it was reported hat fluoxetine has reduced the *defensive behavior* of animals eventually inhibiting 'passive form' and stimulating 'active form' of defensive behavior [68, 70].

Lipid extract Channa Striatus have proven ameliorative effect against depression induced from Chronic Unpredictable Mild Stress Model in rats [71]. Similarly, another report depicted the efficacy of curcumin to alleviate the depression-induced memory defects by modulating oxidative stress & cholinergic activity using chronic unpredictable mild stress-induced depression models [72]. In our study, both N-14 stimulate active forms of defensive behavior exhibiting antidepressant properties. N-14 cotreatment reduced the *aggressiveness* of animals. N-14 also enhanced *defensive behavior* due to the passive form and simultaneously reduced the *sociability* and *exploratory activity* of animals, which is the opposite effect to fluoxetine [https://cyberleninka.ru/article/n/izuchenie-antidepressivnoy-aktivnosti-i-profilya-bezopasnosti-novyh-proizvodnyh-tietan-1-1-dioksida].

In the OF test, it was shown that N-14 cotreatment significantly reduced *Exploratory Activity* and *patterns Moving* and *Sitting* in animals subjected to CMSS compared to Group III. The obtained results have confirmed the reduction in *Social Inactivity* and *Non-Social Exploration*, which was evident from the decrease in exploratory and motor activity of animals treated with N-14 as revealed from the analysis of the RIP.

In groups exposed to CMSS (Groups III and IV), the level of *Exploratory Activity* was higher than in the group of intact animals (Group I). The level of Emotional Anxiety was slightly lower. N14 cotreatment invoked sedative properties evident from the decrease in behavioral patterns of *Exploratory Activity*, *Movement on the Spot*, *and Moving* compared to the stressed rats. Therefore, our results are laying pavement for the future studies to address the molecular and neurobiological signaling underlying the anti-depressive behavioral patterns with N14 in rat models and *in vivo* AD models.

A report by *Sergio D. Iñiguez et al (2014)* delineated that the social defeat stress in adolescent male c57BL/6 mice confer depression like phenotype [73]. The administration of SSR149415 (*a first selective and orally active non-peptide antagonist of vasopressin V1b receptors*) mitigated the physical state of the coat of socially stressed animals during RIP and induced anti-depressant effect [74]. In addition, the findings concluded that SSR149415 administration normalized grooming during CMSS [74]. In our study, EPM tests described an extensive rise in the *Open Arms time*, and reduction in the number of *Open* and *Closed Arms*

entries, as well as an increase in the *number of Closed Arms Returns* and *Head Dippings* over the sides of the maze in the Stress group was observed compared to controls (Group I). These are may be due to manifestation of Risk Assessment behavior rather than an indicator of anxiety in animals [36]. N-14 cotreatment significantly invoked decline in the *Open Arms Time* near normal to intact control animals; however, the number of *Closed Arms Returns* and *Closed Arms entries* substantially increased with N-14 in Group IV compared to both intact and stressed groups. This may indicate a normalization of the behavioral structure of animals subjected to CMSS. Thus, our reports for the antidepressant activity of N14 are in line with above studies.

The reduction in the thymus mass, body weight was evident in rodent models during CMSS-induced depression behavior [75, 76]. CMSS fostered reduction in the weight gain of the Intruders. N-14 cotreatment significantly reduced the weight of Group IV animals and their feed consumption. Interaction between Residents and Intruders led to the increase in mass of thymus of stressed animals (Group III). N-14 reduced the mass of thymus and liver of Intruders in Group IV.

Conclusions

The intake of current anti-depressants has been associated with severe adverse effects in depression patients and AD patients' comorbid with depression. The current study concluded that the dynamic interaction between Residents and Intruders over the course of the experiment showed substantial "increase in exploratory activity (non-social exploration)", "decrease in aggressiveness and defensive behavior", as well as a "decrease in body weight gain" and an "increase in thymus mass" in stressed animals. N-14 cotreatment did not affect the weight gain of the intruders, food consumption. N-14 has proven efficacy to reduce thymus mass in rats subjected to CMSS. N-14 cotreatment has "**reduced** sociability, exploratory activity and aggressiveness" and "**increased** social adaptability and defensive behavior of animals" due to the passive forms of defense with concomitant "**decrease** in exploratory and motor activity. Increased active forms of defense and submission latency indicate that N-14 has antidepressant efficacy with a psycho-sedative component of action; this psychopharmacology study can be further extrapolated for *in vivo* & clinical studies to delineate the molecular signaling for N-14 efficacy.

Limitations

Our study is a preliminary study, previously, the current paradigm has proved as a well established challenging models to study the effects of stress-induced depression despair for the models of social defeat, which characteristically rely on male aggression not readily displayed by female subjects [77-79]. Reports by *Holly et al. 2012; Jacobson-Pick et al. 2013* declared that the social defeat from CMSS models elicit aggressive behavioral displays by resident females; this is making observers very difficult to record the symptoms because the social defeat in female rats appears to invoke several symptoms but only after a delay [49, 79]. Another significant limitation of this study is that we have not determined the influence of anxiety level on the results. Instead we have significantly focused on the predominant differences in the exploratory behaviors between the groups. Our choice of paradigm more broadly focused on to detect the behavioral alterations invoked from the novel anti-depressant molecule as a preliminary step for further future understating of the depression in AD-induced *in vivo* models, and clinical situation that mimics AD-like pathology and behavioral sign .

Future Studies

We will extend our future studies in the forthcoming research project for the understating of the AD-associated depression and underlying pathophysiology, and molecular signaling vividly *in vivo* and clinical models and extrapolate these studies to examine the efficacy of N-14 to mitigate depression-induced behavioral alterations.

Abbreviations

DIM : duration of immobilization

EPM : elevated plus-maze

FST : forced swimming test

OF : open field

CMSS : Chronic mild social stress

Declarations

Availability of data and materials

All data obtained in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The animal experimental procedures were approved by the Ethical Committee for animal care of the Bashkir State Medical University, Ufa, Russian Federation in accordance with the Guidelines on conducting studies involving the use of experimental animals & National Institute of Health Guide for the Care and Use of Laboratory Animals" (NIH Publications No. 80 – 23, revised 1996). The experimental subjects were white outbred rats, males and females. They were kept in

standard vivarium conditions with a 12/12 h light/dark cycle (lights off at 20:00). They had free access to water and food. Cervical dislocation was used to euthanize the animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial and non-financial interests.

Table-1

Effect of N-14 and Social Stress on the behavior of Intruder animals in the Resident-Intruder Paradigm

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Authors' contributions

Irina L. Nikitina (ILN), Kirill V. Bulygin (KVB), and Gjumrakch Aliev (GA) conceptualized and designed the study. ILN, Gulnara G. Gaisina (GGG), KVB, Elmira F. Galimova (EFG), Shamil N. Galimov (SNG), collected and analyzed the data. ILN, GGG, Narasimha M Beeraka (NMB), KVB, EFG, SNG, Vladimir N. Nikolenko (VNN), Liudmila M. Mikhaleva (LMM), Siva G. Somasundaram (SGS), Cecil E. Kirkland CEK), Marco F. Avila-Rodriguez (MAR), NMB, and GA performed the formal analysis, and the results and their interpretation, wrote the original manuscript, revised and improved the original draft. All authors have reviewed and approved the manuscript before submission.

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Figures

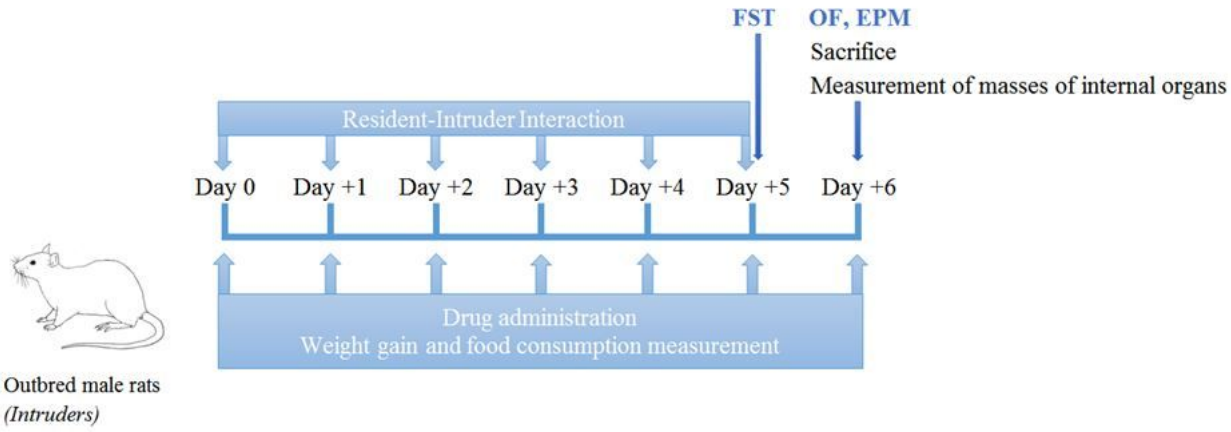

Fig. 1 Scheme of the experimental protocol. Outbred male rats (Intruders, Group IV) received N-14 (2 mg/kg) intraperitoneally on Days 0 to +6. Interaction between Residents and Intruders occurred for 10 minutes daily on Days 0 to +6. On Day +5 Intruders were tested in FST, on Day +6 they were tested in OF and EPM. The weight gain and the amount of food consumed by Intruders were measured during the experiment. At the end of the experiment, the animals were euthanized and the masses of the liver, spleen, thymus, and adrenal glands were measured.

Figure 1

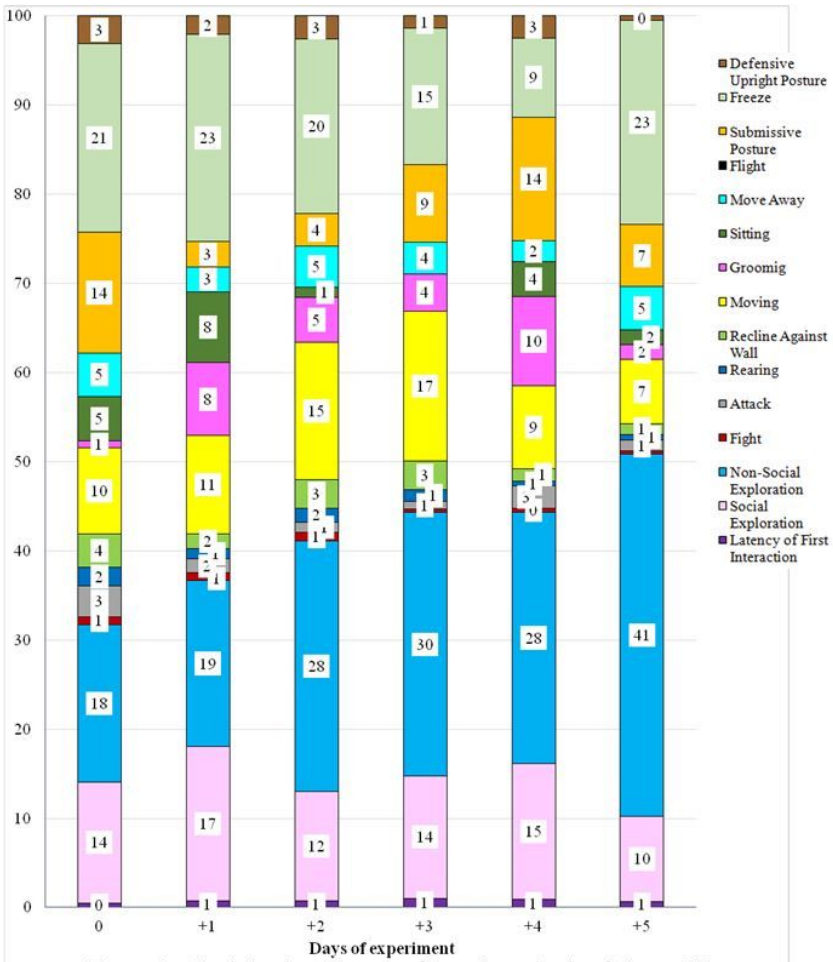

Figure-2: The behavior structure of Intruder animals of Group III received Social Stress on Days 0 to +6 in the Resident-Intruder Paradigm. The proportion of the patterns (%) is given in the bars.

Figure 2

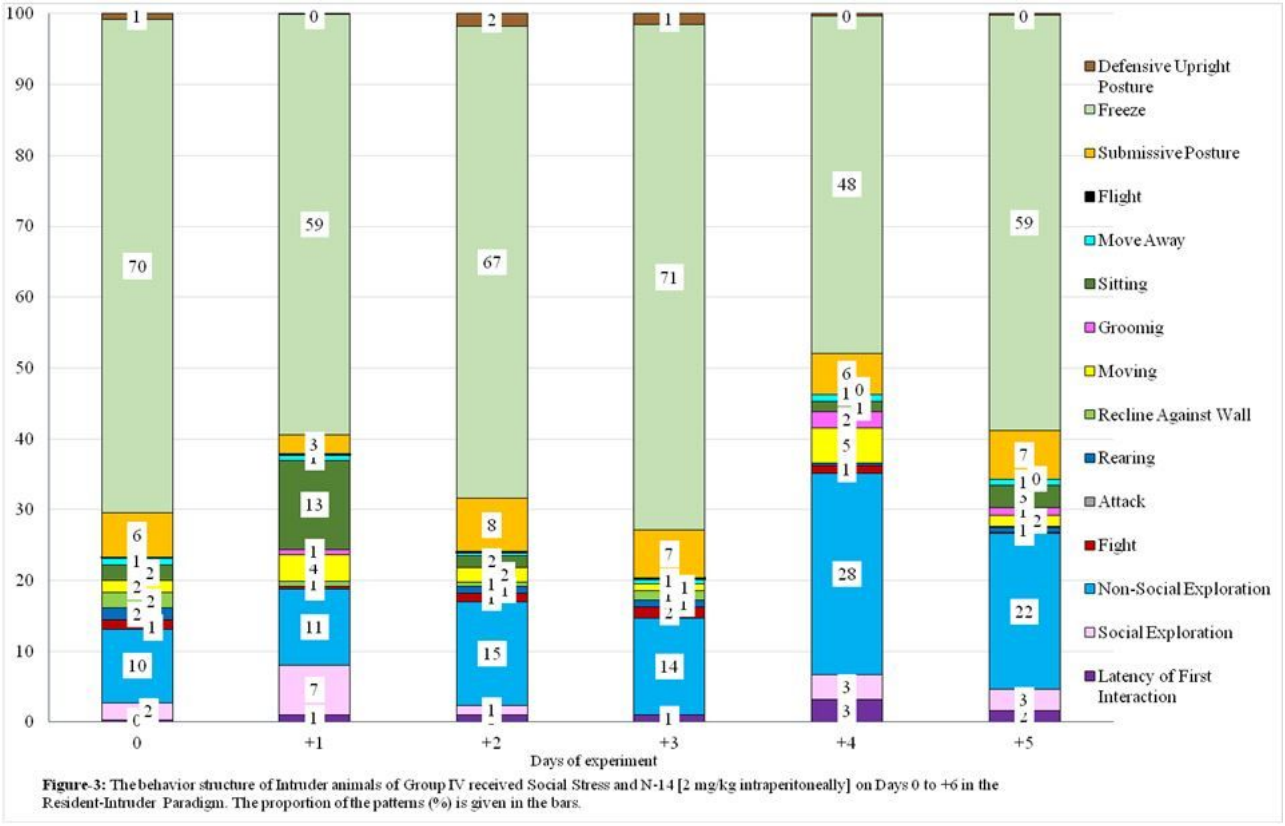

Figure-3: The behavior structure of Intruder animals of Group IV received Social Stress and N-14 [2 mg/kg intraperitoneally] on Days 0 to +6 in the Resident-Intruder Paradigm. The proportion of the patterns (%) is given in the bars.

Figure 3

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