Journal of Global Biosciences
ISSN 2320-1355
Volume 5, Number 3, 2016, pp. 3725-3741
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

PSYCHOPHARMACOLOGICAL ASSESSMENT OF THE SENSORY CONTACT MODEL AS A POSSIBLE MODEL FOR MANIA

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Abstract

Human aggression is a heterogeneous and multifaceted phenomenon, considering its motivation, context, behavioral patterns and presumed goal. The causes and treatment of pathological aggression are poorly understood and understudied. The current study aims to evaluate specific features of the neurobehavioral and biochemical of the aggressive behavior developed in the sensory contact model (SCM) in comparison to resident-intruder paradigm (RIP). Furthermore, the study aims to discuss the possibility to validate SCM as a model of mania in humans. Non agonistic behaviors of the subjects of each model were assessed using open field test (OF) and novel object exploration. In addition, agonistic behavior domains were evaluated in a 10 min session for each model. Neurochemical evaluation of subjects was based on measuring serotonin (5-HT), and its metabolite 5-hydroxy indole acetic acid (5-HIAA), dopamine (DA) and norepinephrine (NE) in brain tissue. Oxidative stress was assessment depended on measuring brain tissue concentrations of reduced glutathione (GSH), nitric oxide (NO), malondialdehyde (MDA) and catalase (CAT) activity. Moreover serum cortisol level was also evaluated. The impact of acute administration of a single dose of lithium chloride (LiCl, 100 mg/Kg i.p) was also studied. Results demonstrated a distinctive difference in the biochemical and the neuro-behavioral pattern of SCM winners compared to that observed in RIP. In conclusion, SCM develops a specific premeditated/pathologic aggressive pattern that seems more correlative to human aggression compared to the instinctive territorial aggression in RIP. In addition, SCM, as a model of dominant-submissive relationship, possibly achieves certain features for face, construct and predictive validity as a model of mania in humans.

Key words: aggression, resident-intruder paradigm, sensory contact model, lithium chloride, mania.

INTRODUCTION

Human aggression is a ubiquitous phenomenon with substantial costs to our society [1]. Aggression per se cannot be considered abnormal; it is one of the most efficient means

of competition that ensures individual survival when resources are limited [2]. Aggression is a heterogeneous and multifaceted, considering its motivation, context, behavioral patterns and presumed goal [3]. The causes and treatment of pathological aggression are poorly understood and understudied. Thus, we have a little knowledge about the biological factors that are associated with pathological aggression in humans [4]. Most of animal research did not focus on the pathological forms of aggression, but rather emphasized on the adaptive ones such as establishing and maintaining dominance in the colony or defending a territory [5]. In addition aggression was rarely considered as an independent disorder in clinical research. Rather, it was usually addressed in terms of psychopathology and was viewed as one of the symptoms that may or may not occur in a particular disorder [6].

A large number of human psychopathologies are usually associated with aggressiveness, namely, Alzheimer's disease, anti social personality, schizophrenia, epilepsy, mania, frontal brain damage, drug abuse…. etc. A recent definition of aggression was established by states that "aggression is a hostile, injurious or destructive behavior often caused by frustration; it can be collective or individual" [7].

Mice are territorial animals that will violently defend their space. The predominant forms of aggressiveness in mice occur in situations of social conflict, when a male defends against a territorial intruder (territorial aggression, inter-male aggression). In preclinical research, aggressive behavior has been predominantly studied by utilizing this natural drive of the animals to defend their territory [8] using the model of resident-intruder paradgim (RIP).

The sensory contact model (SCM) is a model for induction of learned (premediated) aggression [9]. Sensory contact model strongly increases the aggressiveness in male mice and allows aggressive type of behavior to be formed as a result of repeated experience of victory in daily aggressive confrontations. It has been shown that permanent sensory contact enhances aggression in male mice of different strains. For example, 90% to 100% of C57 males are strongly aggressive for 120 to 180 sec in the first confrontation of a 10-min test [10]. Moreover, the totally nonaggressive subjects of CBA strain acquired the performance of the high-aggression strains after a sensory contact period [11]. This suggests that the threshold for the activation of the neural systems for aggression is very low under sensory contact conditions [12]. The current study aims to evaluate the neurobehavioral characteristics of the winners in SCM and also the specific pattern of their aggressive behavior in comparison to RIP, Furthermore, the study aims to discuss the possibility to validate the sensory contact model as a model of mania in humans.

Lithium was the first medication found to have mood stabilizing properties [13], and today remains a first-line treatment for bipolar disorder (BP), manic episodes and suicidal tendencies [14]. However, the actual therapeutic mechanism is not certainly determined yet [15].

MATERIAL AND METHODS

Animals: Swiss albino mice were obtained at 2-3 months of age from the central animal house of the institute), with an initial weight of 20-25 gm. Animals were kept under controlled housing conditions of temperature and humidity and allowed free access to water and conventional diet. Animals were kept together for at least one week before initiation of procedures. The experimental protocol was approved by Institutional Animal Ethics Committee.

Experimental design: animals were randomly assigned into control, RIP, SCM, RIP+LiCl and SCM+LiCl groups. Each of the previously mentioned groups was divided into 2 subgroups, one of them was evaluated for various behavioral parameters and the other was sacrificed to obtain brain and serum for further evaluations.

Treatment: Lithium chloride (LiCl), purchased from Sigma-Aldrich (99%), was dissolved in normal saline and was given as a single dose of 100 mg/Kg i.p. one hour before behavioral evaluation or scarification of the animals.

Resident-intruder paradigm: male mouse was caged with a female, in order to enhance territorial aggression, for 14 consecutive days [16]. At the 15th day, RIP was performed at 15:00-17:00 h. The female partner was removed of the cage and a naive male mouse, i.e. never introduced to a similar paradigm before, of weight equal to the average weight of the resident mice ± 5 gm was introduced to the cage, videotaped for 10 minutes and then removed.

Sensory contact model: the model was described in details elsewhere [10]. In brief, pairs of male mice are placed in cages divided in two compartments by a transparent partition allowing the animals to see, hear and smell their neighbor, but not to contact them physically. Every day the partition was removed for 10 min to allow agonistic interactions. Superiority of one of the partners was evident within 3 daily test sessions with the same partner. One partner attacked, bitted, and chased the other, who displayed defensive behavior only (sideways, upright postures, lying on the back or freezing). Agonistic interactions were discontinued if attacks lasted continuously for more than 3 min. Every day after the test session, each defeated mouse was placed in another two compartments, in which another winner was present in the other compartment. The winners remained in their own compartments.

Control group: group-housed males after 3 days of social isolation were considered as a control group. They were supposed to be best as intact controls since, in this case, the submissiveness of grouped males would be removed, and the repeated experience of aggression would not acquired yet [11].

Behavioral evaluation

Open field test (OFT): The open field was performed according to Weiss and co-workers [17]. In brief, a wooden box (40×40×30 cm) with red walls and white polished bottom and divided into 16 equal squares (10×10 cm each). All experimental sessions were carried out at 16:00-18:00 to minimize the influence of possible circadian changes. Each mouse was placed gently in the middle of the arena and videotaped for 5 minutes. The test period was divided into 2 equal phase, each of 150 sec and were then analyzed for the following parameters: i- Latency: time in seconds elapsed from placement of the subject in the arena till it makes the first move. ii- Ambulation: number of squares the animal entered with all four paws during the test session. iii- Grooming: Time elapsed while the animal was scratching face, licking paws, fur or genitals. iv- Rearing: number of times the animal stands on the hind limb with or without forelimb support. v- Defecation: number of fecal pellets in the open field after the end of the session. vi- percent time spent in the center of the arena: (time in the center/ 150)×100. At the end of the 5 minutes session, the wool puppet was then placed in the open field and the animal was videotaped for further 3 minutes [18], the following parameters were evaluated: i- contact frequency: number of occasions the animal examined the novel object with the nose and/or the fore paws. ii- total exploration time: total time spent in exploring the novel object during examination session. iii- average exploration time: total exploration time/ approaches. After each session, OF was thoroughly wiped using 10% isopropyl alcohol in order to eliminate possible biasing effects due to odor clues.

Agonistic behavior profile: The following behavioral domains were recorded for 10min for the aggressor animals of both models [19]: i- latency of 1st attack: time elapsed till initiation of attack against opponent. ii- frequency of attacks during session. iii- total period of attacks: summation of attacking periods during session. iv- average period of attack: total period of attacks/ frequency of attacks. v- digging: time spent in digging up and scattering bedding in the arena. vi- frequency of tail rattling made by the observed animal during session. vii- aggressive grooming: total time in which observed animal mounting the defeated animal's back, holding it down, and spending much time licking and nibbling at the scruff of the defeated male's neck.

Neurochemical evaluation: brain dopamine (DA), norepinephrine (NE), serotonin (5-HT) and its metabolite 5-hydroxy indol acetic acid (5-HIAA) were determined spectrophoto-florometrically [20] using spectro-photoflourometer (RF-5000 Shimadzu, Japan). Animals were decapitated with the least disturbance and brain was rapidly dissected on ice, weighed and stored at -80 °C for later evaluation.

Biochemical evaluation

Catalase (CAT) Activity: tissue catalase was determined according to the method of Evans and Diplock [21]. Brain homogenates were diluted with buffer then, the diluted solution was mixed with 30 mmol/L H2O2. Absorbance at 240 nm was measured for 100 seconds. The enzyme activity (U/g tissue) is expressed as the first order constant that describes the decomposition of H2O2 at room temperature. Tissue protein content was estimated for calculation [22].

Reduced glutathione (GSH) content: Brain tissue was homogenized in an ice-cold 150 mol/L KCl for determination of GSH levels. A spectrophotometeric method using Ellman's reagent [23] was used for determination of GSH content. Absorbance was measured within 5 min at 412 nm using a spectrophotometer (Heλios a theromospectonic). Results were expressed as µmol/g tissue.

Lipid Peroxidation: Brain lipid peroxidation was assessed by determining the malondialdehyde (MDA) content of tissue homogenates using a colorimetric assay [24]. Briefly, to 0.5 ml tissue homogenate, 1 ml of 20% trichloroacetic (TCA) was added. After precipitating the protein with TCA, 3ml of 1% orthophosphoric acid (1% H3PO4) and 1 ml of 0.6% thiobarbituric acid (0.6 TBA) were added and then incubated in a boiling water bath for 40 min. After cooling, the samples were extracted with n-butanol and centrifuged at 4000 rpm. The absorbance of samples was determined at 520 and 535 nm. Concentrations of MDA were expressed as nmol/g tissue.

Determination of nitric oxide (NO) concentration: Total nitrate/nitrite accumulation in brain was performed as an indication of NO production [25]. The procedure was based on the reduction of nitrate by vanadium (III) chloride (VCl3) combined with detection by Griess reaction. The absorbance at 540 nm was measured using a spectrophotometer (Heλios α thermospectonic).

Evaluation of serum cortisol level: Serum cortisol level was determined using enzyme-linked immunosorbent assay (ElISA) assay (dbc, the EiAsy way). The assay was performed according to the manufacturer's instructions, and read at 405 nm using ELISA reader (BioTEk Instruments Inc., ELx 808, USA). Concentration was expressed as µg/dl.

Statistical analysis: data were analyzed using either one way ANOVA followed by least significant difference test for parametric parameters or Kruskal-Wallis test followed by Mann-Witney U test and Wilcoxon rank sum test for non-parametric ones. All Statistical procedures were performed using SPSS 17 computer package.

RESULTS

Behavior of RIP subjects in the open field (OF) and effect of LiCl

Subjects of RIP group demonstrated a significant increase in ambulation ($p \leq 0.05$) during both phases (109% and 125%) and rearing (63% and 26%) accompanied by a corresponding significant decrease in grooming time ($p \leq 0.05$) at both phases (57% and 43% respectively). Moreover, RIP subjects spent longer time in the center of the arena (phase 1: 178%, phase 2: 440%, $p \leq 0.05$) compared to control subjects (table 1). Administration of LiCl resulted in amelioration of the hyper-locomotion of RIP subjects manifested by significant decrease ($p \leq 0.05$) of ambulation at both phases (42% and 82% respectively) and rearing frequencies (63%, 81%). Subjects given LiCl spent less time in the center of the arena (phase 1: 67%, phase 2: 93%) with a corresponding longer time spent in grooming (phase 2: 284%) compared to untreated group (table 1).

Behavior of SCM subjects in the open field (OF) and effect of LiCl

A significant increase ($p \leq 0.05$) in ambulation frequency during phase 2 (33%) correlated with significant decrease in rearing during both phases by 50%, 35% respectively (table 1). This was accompanied by a significant increase ($p \leq 0.05$) in the latency by 91% and a corresponding abolishment of defecation frequency (100%). SCM subjects spent significantly ($p \leq 0.05$) less time in the center of the arena during phase 1 (58%) but longer time (388%) during phase 2 as compared to control group (table 1). Subjects of SCM given LiCl expressed a significant decrease ($p \leq 0.05$) in ambulation and rearing frequencies and time spent in the center during phase 2 only (41%, 63% and 81% respectively. Moreover, LiCl resulted in a significant increase ($p \leq 0.05$) in the defecation frequency (200%) and phase 2 grooming time (565%).

Table 1: Effect of LiCl on the behavior of RIP and SCM subjects in the open field (OF) and novel object exploration

Behavior in OF	Control	RIP	SCM	RIP + LiCl	SCM + LiCl
Latency	2.91 ± 0.525	3.93 ± 0.685	5.56 ± 1.4	1.5 ± 0.53 d (- 62%)	3.25 ± 0.62
Ambulation phase 1[*]	42.5 (38-48)	89 (65-103) a (109%)	49 (32-73) b (-45%)	52 (42-62) d (-42%)	60 (21-83)
Ambulation phase 2[*]	36 (26-47) e (- 15%)	81 (59-87) a (125%)	48 (41-80) a (33%), b (-41%)	14.5 (10-30) d (-82%) e (-72%)	28.5 (10-45) d (-41%) e (-53%)
Rearing phase 1[*]	16 (6-20)	26 (15-34) a (63%)	8 (0-15) a (-50%) b (-69%)	9.5 (6-14) d (-63%)	7.5 (2-20)
Rearing phase 2[*]	23 (13-27) e (44%)	29 (25-39) a (26%)	15 (3-22) a (-35%), b (-48%), e (87%)	5.5 (3-7) d (-81%)	8.5 (3-13) d (-43%)
Grooming phase 1	9 ± 0.6	3.9 ± 0.88 a (-57%)	4.22 ± 1.32 a (-53%)	6.5 ± 0.85	4.62 ± 1.08
Grooming phase 2	11.5 ± 1.74	6.57 ± 2.35 a (-43%)	5.37 ± 0.5 a (-53%)	25.25 ± 2.18 d (284%) e (288%)	35.7 ± 5.54 d (565%) e (673%)
Time in the center ph 1	5.34 ± 0.64	14.85 ± 1.9 a (178%)	2.22 ± 0.547 a (-58%) b (-85%)	4.87 ± 0.64 d (-67%)	2.12 ± 0.74

Time in the center ph 2	2.34 ± 0.4 e (-56%)	21.83 ± 3.88 a (833%) e (47%)	11.43 ± 1.13 a (388%), b (-48%), e (415%)	1.5 ± 0.422 d (-93%) e (-69%)	2.125 ± 0.48 d (-81%)
no. of fecal pellets[*]	1 (0-3)	2 (0-4)	0 a (-100%) b (-100%)	0.5 (0-2)	3 (0-6) a (200%)
Contact frequency [*]	8.5 (3-15)	13 (11-16) a (53%)	4 (3-7) a (-53%) b (-69%)	1.5 (1-3) d (-88%)	4.5 (2-7)
Total contact time	68.86±5	82.86±3.62	104.1±5.38 a (51%), b (26%)	2.71±0.64 a (-96%) d (-97%)	41.9±8.13 d (-60%)
Average contact time	7.41±0.57	6.21±0.26	24.42±1.85 a (230%) b (293%)	2±0.38 a (-73%) d (-68%)	9.4±0.82 d (-61%)

Data expressed as mean ± SEM and percent change from the corresponding significant group, n=8. Statistical analysis was carried out using one way ANOVA followed by least significant difference test. [*]: Data expressed as median ± range, n=8. Statistical analysis was carried out using Kruskal-Wallis followed by Mann-Whitney U test. a: significant difference from control ($p \leq 0.05$). b: significant difference from RIP ($p \leq 0.05$). d: significant difference from corresponding untreated group ($p \leq 0.05$). e: significant difference from phase 1 ($p \leq 0.05$).

Exploratory behavior of a novel object and effect of LiCl

Subjects of RIP demonstrated a significant increase ($p \leq 0.05$) in the frequency of contact with novel object (53%). LiCl administration resulted in abolishment of the exploratory tendency of RIP group manifested by significant decrease ($p \leq 0.05$) in the frequency, total and average time of contact (88%, 97% and 68% respectively) when compared to untreated group (table 1). On the contrary, SCM group expressed an extensive interest in the novel object evidenced by the significant increase ($p \leq 0.05$) in both total and average contact time (51% and 230%) respectively but not contact frequency as compared to control group. LiCl modulated the exploratory behavior of SCM group in a certain manner, as it was able to restore both total and average contact time into control values (table 1).

Agonistic behavior profile of RIP subjects and effect of LiCl

Resident subjects expressed a significantly short latency ($p \leq 0.05$) before attacking intruder compared to controls by 89%. The agonistic behavior of residents against intruders was direct and impulsive during phase 1 but rather subsides rapidly during phase 2, evidenced by significant decrease ($p \leq 0.05$) in total attack time, attacks frequency (24%), tail rattlings (57%) and biting frequencies (47%). Furthermore, resident mice demonstrated an indirect aggression towards intruder during phase 2, manifested by significant increase ($p \leq 0.05$) of aggressive grooming (43%) and digging (611%) (table 2). LiCl administration resulted in a significant decrease ($p \leq 0.05$) attacks frequency (24%) with a corresponding increase in aggressive grooming during phase 1 (420%) as compared to untreated group (table 2).

Table 2: Effect of LiCl on the agonistic behavioral profile of RIP and SCM subjects.

Agonistic behavior	Control	RIP	SCM	RIP + LiCl	SCM + LiCl
Latency	41.88±3.4	4.75±0.59 a (-89%)	1.75±0.25 a (-96%) b (-63%)	6.63±0.65	36.75±4.76 d (2000%)
Total attack time	13.65±1.85	50.88±5.2 a (273%)	121.75±7.47 a (792%) b (140%)	46.75±2.8	42.75±5.48 d (-65%)
Average attack time	1.95±0.06	2.3±0.102	4.2±0.085 a (115%) b (83%)	2.29±0.121	4.16±0.146
Attack frequency ph 1[*]	3 (1-5)	12.5 (7-22) a (317%)	12.5 (9-17) a (317%)	9.5 (7-12) d (-24%)	7 (3-10) d (-44%)
Attack frequency ph 2[*]	4 (1-6)	9.5 (4-18) a 138%, b 68% e 24%	15.5 (13-20) a 288%, b 48% e 63%	11 (7-14) a 175%	3.5 (2-6) d 77%
Tail rattling ph 1[*]	0	10.5 (4-12) a (1050%)	7.5 (6-10) a (750%)	8.5 (4-10) a (850%)	2 (0-3) d (-73%)
Tail rattling ph 2[*]	1 (0-2)	4.5 (2-7) e (-57%)	9 (4-11)	3.5 (1-6) e (-59%)	1 (0-2) d (-89%)
Biting frequency ph 1[*]	0	17 (10-20) a (1700%)	11 (8-14) a (1100%) b (-30%)	13 (9-17)	2 (1-3) d (-82%)
Biting frequency ph 2[*]	0.5 (0-1)	9 (6-12) a (850%) e (-47%)	13.5 (11-17) a (2600%)	8.5 (4-10) a (800%) e (-35%)	1 (0-3) d (-93%)
Digging ph 1	3±0.72	8.5±0.87 a (183%)	11.38±0.94 a (280%) b (34%)	7.13±0.64	10.25±0.8
Digging ph 2	4.5±0.8	12.13±1.15 a (170%) e (42%)	23.13±1.43 a 412%, b 91% e 103%	9.88±0.85 e (38%)	4.81±1.7 e (-53%)
Grooming ph 1	7.63±1.48	2.25±0.675 a (-70%)	25.9±1.87 a (240%) b (1051%)	11.75±0.75 d (422%)	26.25±2.05
Grooming ph 2	2.25±0.6	16±1.43 a (611%) e (611%)	66.25±3.65 a (2840%), b (314%), e (156%)	18±0.95 e (53%)	63.4±3.7 e (141%)

Data expressed as mean ± SEM and percent change from the corresponding significant group, n=8. Statistical analysis was carried out using one way ANOVA followed by least significant difference test. [*]: Data expressed as median ± range, n=8. Statistical analysis was carried out using Kruskal-Wallis followed by Mann-Whitney U test. a: significant difference from control ($p \leq 0.05$). b: significant difference from RIP ($p \leq 0.05$). d: significant difference from corresponding untreated group ($p \leq 0.05$). e: significant difference from phase 1 ($p \leq 0.05$).

Agonistic behavior profile of SCM subjects and effect of LiCl

SCM subjects expressed the shortest latency of attack compared to either control (96%) or RIP (63%) groups, correspondingly with the significantly longer ($p \leq 0.05$) average attack time (97% and 83% respectively). In addition, SCM group demonstrated an escalating aggressiveness during phase 2, manifested by the significant increase ($p \leq 0.05$) of both direct (attack frequency: 40%, total attack time: 45%) or indirect (digging:

103%, aggressive grooming: 156%) compared to phase 1 (table 2). Subjects of SCM expressed a typical response upon LiCl administration. Single dose of LiCl blunted the direct aggressiveness toward opponent, namely, latency of attack (2000%), total attack time (65%), attack frequency (phase 1: 44%, phase 2:77%), tail rattlings (phase 1: 73%, phase 2: 89%) and biting frequency (phase 1:82%, phase 2: 93%). However, LiCl failed to alter either average attack time, digging or aggressive grooming during both phases.

Neurochemical profile of RIP subjects and effect of LiCl

A significant decrease (p≤ 0.05) in 5-HT (11%) and its metabolite 5-HIAA (54%) was observed as compared to control group. This was accompanied by a subsequent decrease in 5-HIIA/5-HT ratio (45%) and a corresponding significant decrease (p≤ 0.05) of NE level (11%) compared to control group. On the other hand, a significant increase (p≤ 0.05) in DA concentration (63%) was observed (fig. 1, 2, 3). Administration of LiCl resulted in a further decrease (p≤ 0.05) in 5-HT level (15%) accompanied by significant increase (p≤ 0.05) of 5-HIAA (200%) and 5-HIAA/5-HT ratio (233%) as compared to untreated group. Moreover, acute administration of LiCl resulted in a significant decrease (p≤ 0.05) in DA concentration (39%) and a corresponding increase of NE (18%) to reach normal levels of control group.

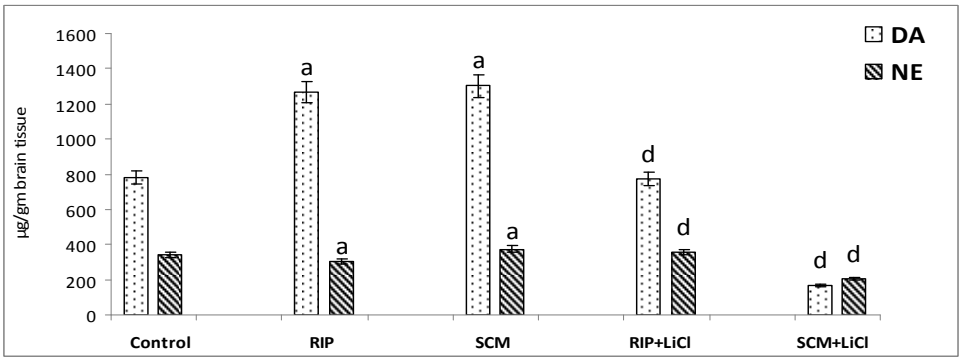

Figure (1): Influence of LiCl (100 mg/Kg i.p.) on the brain tissue content of dopamine (DA) and norepinephrine (NE) of the RIP and SCM subjects. Data are expressed as mean ± SEM, n=7. a: significant difference from control (P ≤ 0.05). d: significant difference from corresponding untreated group (P ≤ 0.05).

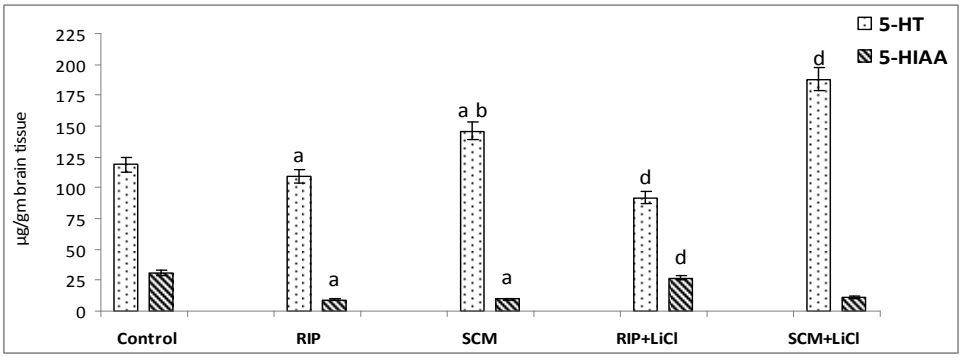

Figure (2): Influence of LiCl (100 mg/Kg i.p.) on the brain tissue content serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) of the subjects of RIP and SCM. Data are expressed as mean ± SEM, n=7. a: significant difference from control (P ≤ 0.05). b: significant difference from RIP (P ≤ 0.05). d: significant difference from corresponding untreated group (P ≤ 0.05).

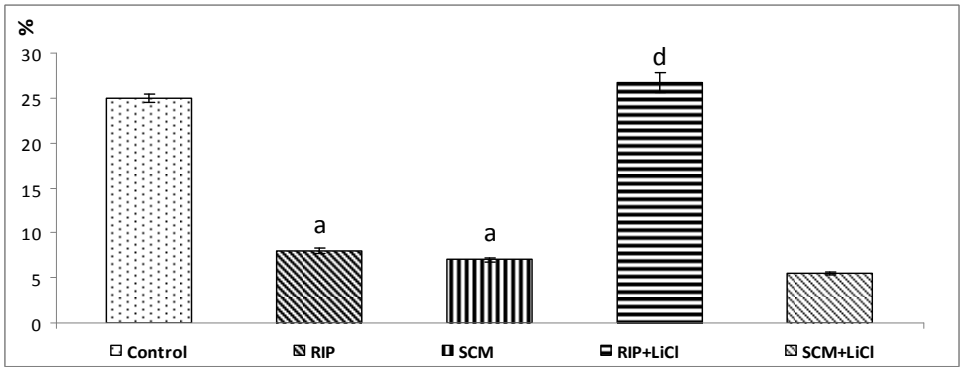

Figure (3): Influence of LiCl (100 mg/Kg i.p.) on the 5-HIAA/5-HT ratio of RIP and SCM subjects.
Data are expressed as mean ± SEM, n=7. a: significant difference from control (P ≤ 0.05). d: significant difference from corresponding untreated group (P ≤ 0.05).

Neurochemical profile of SCM subjects and effect of LiCl

Subjects of SCM demonstrated a significant increase (p≤ 0.05) of 5-HT level (22%) correlated with a significant decrease (p≤ 0.05) of 5-HIAA level and 5-HIAA/5-HT ratio (69% and 73% respectively. This was accompanied by a remarkable activation of chatecholaminergic system evidenced significant increase (p≤ 0.05) of both DA and NE levels (67% and 10% respectively) when compared to control group. Single dose of LiCl resulted in a further increase of 5-HT (29%) but not 5-HIAA leading to a significant decrease (p≤ 0.05) in 5-HIAA/5-HT ratio (20%) as compared to untreated SCM subjects. On the other hand, LiCl resulted in an acute suppression of the chatecholaminergic activity expressed by significant decrease 5-HIAA/5-HT ratio of DA (87%) and NE (45%) concentrations when compared to untreated subjects (fig. 1, 2, 3).

Antioxidants/oxidative stress state of RIP subjects and effect of LiCl

RIP group demonstrated significantly higher level (p≤ 0.05) of GSH (23%) in brain tissue (fig. 8) with a corresponding decrease in NO concentration (20%) compared to control group (fig. 11), while both MDA concentration and catalase activity was kept unchanged (fig. 5, 6). LiCl resulted in further decrease (p≤ 0.05) of NO (15%) that was accompanied by a significant decrease (p≤ 0.05) of GSH level (17%) (fig. 4, 7). On the other hand, LiCl administration significantly increased (p≤ 0.05) brain MDA content (54%, fig. 10). However, LiCl did not alter catalase activity (fig. 5).

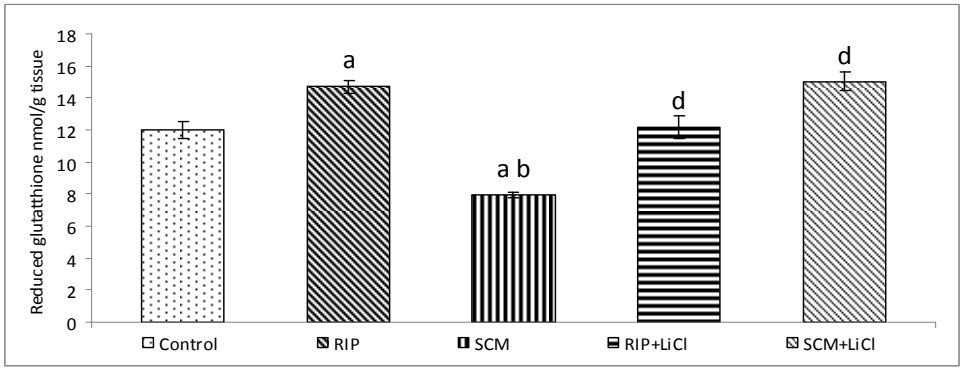

Figure (4): Influence of LiCl (100 mg/Kg i.p.) on the glutathione (GSH) content in the brain tissue of the subjects of RIP and SCM. Data are expressed as mean ± SEM, n=7. a: significant difference from control (P ≤ 0.05). b: significant difference from RIP (P ≤ 0.05). d: significant difference from corresponding untreated group (P ≤ 0.05).

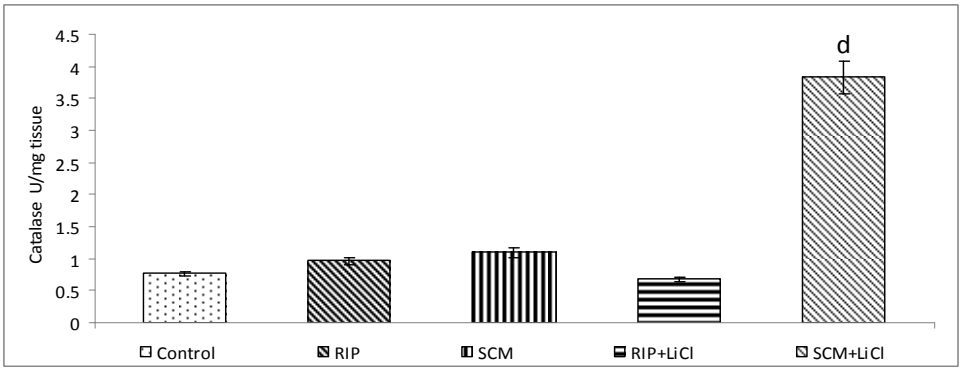

Figure (5): Influence of LiCl (100 mg/Kg i.p.) on the catalase (CAT) activity in the brain tissue of the subjects of RIP and SCM. Data are expressed as mean ± SEM, n=7. d: significant difference from corresponding untreated group (P ≤ 0.05).

Antioxidants/oxidative stress state of SCM subjects and effect of LiCl

SCM subjects demonstrated a significant decrease (p≤ 0.05) of both GSH and NO concentrations (34% and 18% respectively) in brain tissues (fig. 4, 7), correlated with a corresponding increase in MDA concentration (120%, fig. 6). Administration of LiCl resulted in restoring oxidative balance, as it significantly increased (p≤ 0.05) GSH level (90%) correspondingly with suppression of MDA concentration (41%). Moreover, LiCl triggered an remarkable activity of catalase (520%) in the examined subjects (fig. 5).

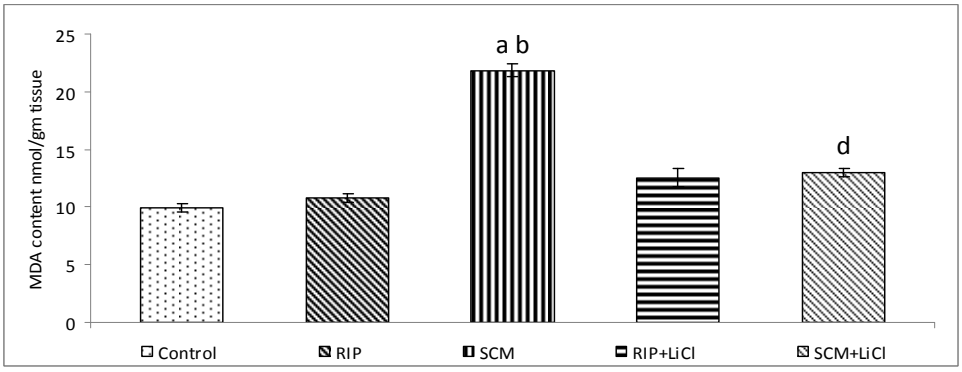

Figure (6): Influence of LiCl (100 mg/Kg i.p.) on the malondialdehyde (MDA) content in the brain tissue of the subjects of RIP or SCM. Data expressed as mean ± SEM, n=7. a: significant difference from control (P ≤ 0.05). b: significant difference from RIP (P ≤ 0.05). d: significant difference from corresponding untreated group (P ≤ 0.05).

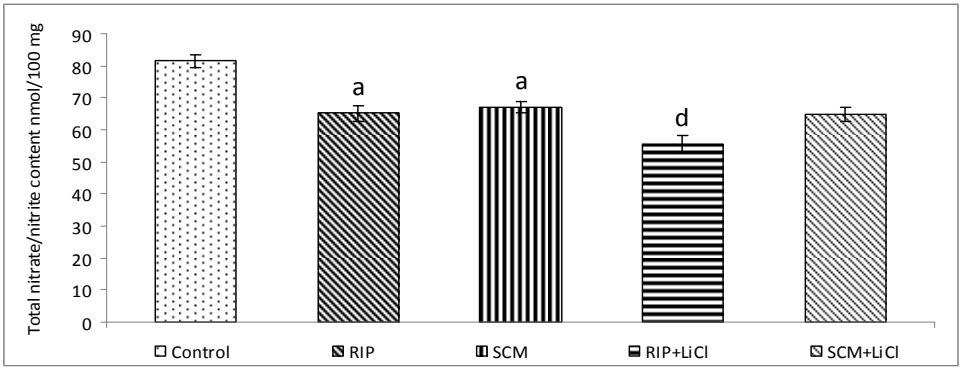

Figure (7): Influence of LiCl (100 mg/Kg i.p.) on the total nitrate/nitrite content in the brain tissue of the subjects of RIP and SCM. Data are expressed as mean ± SEM, n=7. a:

significant difference from control (P ≤ 0.05). d: significant difference from corresponding untreated group (P ≤ 0.05).

Serum cortisol level and effect of LiCl

A significantly high level (p≤ 0.05) of serum cortisol level was observed in RIP subjects (144%) that was significantly lowered (p≤ 0.05) towards normal control values (46%) in response to LiCl administration (fig. 8). On the other hand, SCM did not express any significant change in serum cortisol level compared to control group. However, LiCl significantly increased (p≤ 0.05) serum cortisol concentration (400%) when administered to SCM subjects (fig. 8).

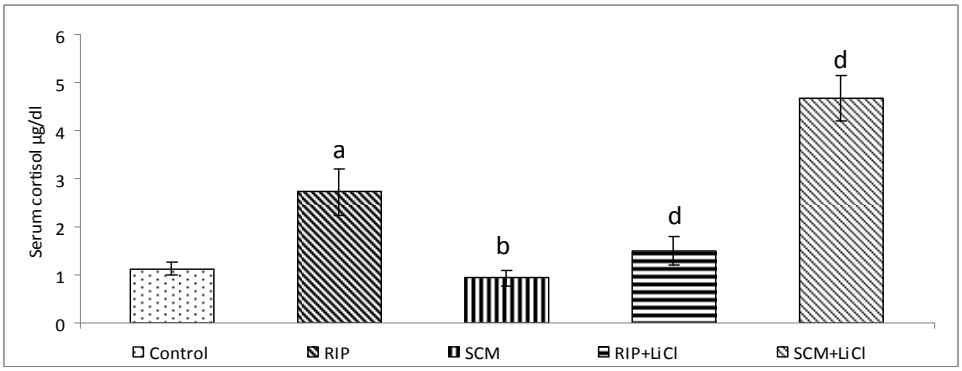

Figure (8): Influence of LiCl (100 mg/Kg i.p.) on the serum cortisol level of the subjects of RIP or SCM.

Data are expressed as mean ± SEM, n=7. a: significant difference from control (P ≤ 0.05). b: significant difference from RIP (P ≤ 0.05). d: significant difference from corresponding untreated group (P ≤ 0.05).

DISSCUSION

Animal behavior in the open field is rather complex as it involves neophobia, arousal and exploration. The balance between them is directly modulated by the emotional state of the animal and its behavioral and physiological activity [26]. In the current study, RIP subjects expressed a hyper-locomotive response (ambulation, rearing) and escalated exploratory activity (rearing and frequency of contact with novel object). Moreover, RIP subjects spent longer time in the center of the arena as a sign of decreased anxiety. Studies pointed to the ability of RIP induced aggression to relief depressive-like behaviors in male rats subjected to chronic mild stress [27]. On the other hand, SCM subjects expressed a state of pronounced anxiety in OF, evidenced by the significant decrease in the time spent in the center of the OF compared to either control or RIP subjects. The state of anxiety persist upon exposure to the elevated plus maze (data not shown). In addition, they expressed a state of decreased emotionality manifested by abolishment of defecation. There is a possible relationship between pathologic aggression and anxiety in laboratory animals, males of the more aggressive strains have higher levels of anxiety than low aggressive ones [28]. SCM subjects expressed a state of diminished exploratory motivation, manifested by decrease in both ambulation and rearing frequencies during both phases in addition to the decreased in the frequency of contact with a novel object. However, they demonstrated prolonged total and average contact time when compared to other groups. The lack of exploratory tendencies is regarded as a sign of impaired adaptation to novelty and stressors [19]. Freezing in OF is considered an adaptive reflex in unavoidable or inescapable frightening conditions [29]. Moreover, the prolonged examination of the novel object

(more than 24 sec/contact) points to the development of pathological forms of behavior [11].

Territorial aggression and inter-male aggression are the predominant forms of aggressiveness in mice occur in situations of social conflict, when a male defends against a territorial intruder [8]. Resident subjects demonstrated an instant hostile response against naïve intruders in the 1st phase of observation that was significantly declined in the 2nd; a predictable pattern based on the defensive motivations lying behind the animal response. The agonistic behavioral pattern during phase 1 was characterized by increment of direct agonistic actions (number of attacks, biting of vulnerable targets and tail rattling) while emphasized on ritual ones (digging, aggressive grooming) in the second one. The brief average attack time (2 sec.) points to lack of interest in attacking the opponent once demonstrating signs of submission (vocalization, immobility, flee).

Concerning SCM, Repeated daily victories for 20 consecutive days resulted in a unique pattern of agonistic response. Subjects possessed shorter latency of 1st attack along with longer total attack time as compared to RIP group. A worth noting that SCM subjects demonstrated a prolonged average attack time accompanied by an increased biting frequency and demonstrated a continuous high level of direct agonistic actions during both phases and even an increasing indirect one during phase two.

The main features of the aggressive profile of SCM subjects could be clearly observed; first, the high average attack time that points to a state of pathological aggression as the dominant subjects continue vigorous attacks, with biting targeting vulnerable areas, against the subordinate conspecifies in spite of the full submissiveness expressed by the later [11]. Second, the emergence of novel "ritual" expression of dominance upon defeated subjects manifested by indirect agonistic actions such as digging and aggressive grooming of the opponent [30]. Third, the lowered threshold of aggressiveness and increased impulsivity, as expressed by the decreased latency of 1st attack compared to RIP subjects. Fourth, Hypersensitivity and inadaptability, subjects demonstrate inadequate behavioral response towards fully subordinate opponent continuously during the whole session with provocation of abnormal aggressive burst for the least stimuli and even towards female subjects [31].

Instead of the adaptive agonistic response of RIP subjects, SCM winners learned to express ritual form of indirect aggression toward the submitting partner (aggressive grooming and digging) that seems "humiliating" and requires less physical effort than direct agonistic action [11]. It should be noticed that such behavior (aggressive grooming) does not arise from an emotional response as evidenced by the lack of emotionality expressed by the winners in the open field [9].

Serotonin has an important role in modulating the activity of the prefrontal cortex and therefore in inhibiting aggressive behavior [32]. Thus, inhibition of the serotonergic system in RIP group is probably correlated with agonistic behavior. The increased DA level observed is in consistent with previous studies [33]. The low intracellular NE levels could probably result from the activation of the sympatho- adrenomedullary system [34]. An unexpected increase in 5-HT level accompanied by a concurrent decrease in 5-HIAA concentrations and 5-HIAA/5-HT ratio was found in SCM group compared to control one. It could be postulated that SCM suppress serotonergic system through attenuation of tryptophan hydroxylase (TPH), the rate limiting enzyme of 5-HT biosynthesis, and inhibition of 5-HT release leading to its intracellular accumulation and inducing a negative feedback inhibitory mechanism [35].

Although studies pointed to a direct relationship between aggressiveness in RIP and the level peripheral intracellular ROS [36], the current study was unable to reproduce these results considering GSH, catalase or MDA levels in brain tissue. This could possibly due to strain difference and/or applied techniques. On the other hand, the significant increase in serum cortisol level results from the activation of HPA axis and is considered as an expression of increased ACTH levels [37]. The oxidative stress state of SCM group points to its peculiar nature. Unlike those RIP, the experienced subjects of SCM did not suffer any stress during agonistic confrontations as evidenced by the normal cortisol level. Moreover, SCM subjects expressed decrement of total nitrates level along with low GSH concentration correspondingly with high MDA level. The major part of brain total nitrates arises from nitric oxide, which is considered as an atypical antioxidant rather than a pro-oxidant [38]. It plays a major role in the protection of brain DA neurons against oxidant stress and damage caused by reactive OH^- species [39] generated during the hydroxylation of L-tyrosine to L-DOPA via tyrosine hydroxylase, the rate-limit step for dopamine biosynthesis [40]. This depleting pathway, considering the elevated DA concentrations measured, results in the lack of NO moiety required to react with GSH to obtain 5-nitrosoglutathione (GSNO), a 5 times more potent antioxidant than glutathione [41]. As a result, GSH levels are overwhelmed by the accumulated toxic adducts of lipid peroxidation (MDA) leading to cell apoptosis and/neurotoxicity [42].

In the current study, lithium-treated subjects of both models performed poorly in the OFT. Significant reduction of either indirect measurements of exploration and novelty seeking (ambulation, rearing and time spent in the center of the arena) or direct ones (number of contacts, total and average contact time with a novel object) accompanied by an increased emotionality, expressed by increased self grooming, was demonstrated. Lithium potential to suppress hyper locomotion in the open field, as well as exploratory behaviors, is well established [43] and could possibly considered as a direct association of the diminished aggressiveness. Resident subjects demonstrated a typical response upon LiCl administration, as it normalized both DA and NE to control levels, this was accompanied by a decrease of intracellular 5-HT with a concurrent increase of its metabolite 5-HIAA, indicating a flux release of 5-HT. Such effect of LiCl lead to a attenuation of aggressiveness against intruders, evidenced by the decreased attack frequency, total attack time, biting, digging and tail rattling during both phases of observation. Studies elucidated similar effect of LiCl [44]. Upon evaluating the state of brain oxidative stress, the most significant observation was the ability of LiCl to decrease MDA level. Although the mechanisms by which lithium modulates ROS are poorly understood, studies postulated that lithium might exert such effect through buffering Ca^{2+} levels and/or stabilizing mitochondrial functions [45]. It could be inferred that such decrement in ROS levels arises from the ability of Lithium to relief the stress induced by resident-intruder situation [46]. On the other hand, LiCl administration modulated the aggressive behavioral profile of SCM subjects in a specific manner, expressed by total suppression of both direct and indirect aggressive behaviors. Aggressive grooming was an exception, probably as a result of the previously mentioned increment of emotionality induced by lithium. Such impact on aggressiveness correlates with suppressing the excitatory chalecolaminergic (NE and DA) systems, as it resulted in an acute suppression of its concentrations rather than restoring it to normal control values as in RIP group. The potential of lithium against amphetamine induced hyperactivity and neuroleptics induced super sensitivity, might explain the capacity of lithium to lower DA levels [47]. The competency of lithium to increase 5-HT concentration is merely related to its positive influence on brain

tryptophan uptake [48]. In addition, studies pointed to the reduction in NE concentration upon administration of lithium [49]. Furthermore, LiCl augments the antioxidant capacity in brain tissue via significant elevation of both GSH and CAT levels resulting in a corresponding decrease of MDA. These findings are in consistent with previous studies [50]. The current results support the hypothesized ability of lithium to reduce pathological aggression [48].

In contrast to mood depression, abnormal mood elevation are not easily modeled in animals, the majority of tests used to date based on the increment of locomotor activity as a measure of manic-like activity [51]. Several ethological models were adapted to study manic episodes, including sleep deprivation, amphetamine-induced hyperactivity and RIP. However, these models fail to perturb animal behavior in a way equates to mania [52]. Recently, dominant-submissive relationship has been proposed to model symptoms of mania and depression respectively [53]. Dominant animals, similar to manic state in humans, are characterized by self-confident, assertive and aggressive behavior [54].

The obtained results might be useful in establishing the validity of SCM as a model for mania. Common features between dominant subjects in SCM and manic episodes in human include uncontrollable aggression (rage), hyperactivity, irritability, inadaptability and disturbances in social and sexual recognition, all supports face validity. In addition the neurochemical state of diminished inhibitory pathways (5-HT) in the favor of excitatory ones DA and NE) establish for construct validity. Moreover, the typical response of SCM subjects to lithium, the gold standard for treatment of BPD, augments the predictive validity of the model. The validation of SCM as a model for mania needs further extensive investigations that might require certain technical modifications of the model.

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