Original Article

Impact of Sensory Contact Model on Psychosocial Stress and Correlation with Immunological Changes

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Abstract

Background and objectives: Chronic stress plays a central role in the pathogenesis of psychiatric disorders. A sensory contact model induces mixed anxiety/depression-like behaviors. Repeated experience of victories or defeats may change neurophysiological status, the immune system and brain neurochemistry in opposite directions. The objective of this study was, therefore, to establish a sensory contact model for studying the impact of psychosocial stress in mice and investigate its influence on behavioral, neurochemical and immunological changes of both winners and losers.

Methods: Four groups of male mice were used, including two groups that received saline and either in normal housing or caged individually for 5 days; the others were subjected to sensory contact modeling for 21 days. All mice were subjected to open-field test, after which blood samples were collected for evaluation of total and differential leukocyte count and brain homogenate was used to estimate monoamines.

Results: Social isolation reduces serotonin and neutrophils while elevating most other parameters. Winners also showed reduction in serotonin and neutrophils, as compared to controls which showed reduction in grooming time, total leukocyte count, neutrophils associated with elevation in monocytes and eosinophils as compared to isolation group. On the other hand, losers showed elevation in grooming time, dopamine, norepinephrine, lymphocytes, monocytes, eosinophils associated with reduction in ambulation, serotonin and neutrophils as compared to all groups. They also showed reduction in rearing and elevation in total leukocyte count as compared to winners.

Conclusions: Social stress leads to severe depression and anxiety-related behaviors; losers were more depressed than winners. However, aggressive behavior was increased in winners, while locomotor and exploratory activities were decreased, indicating decreased anxiety and emotional distress. The immune function was enhanced to higher extent in losers than winners, which can be attributed to sensation of threat and trauma in losers.

Introduction

Stress plays a leading role in a number of psychiatric disorders, functional disorders of the gastrointestinal tract, autoimmune diseases, coronary heart disease, chronic pain and several other medical disorders.[1,2] Anxiety and depression are considered as common mental disorders, but the mechanisms through which chronic stress increases their vulnerability are unclear. However, it is now becoming clear that without knowledge of both clinical and biological aspects of anxiety and depression, it is difficult to offer effective treatment strategies for patients.[1,3] According to McKinney,[4] we use animal models as "experimental preparations developed in one species for the purposes of studying phenomena occurring in another species".

Social defeat, which is the result of intraspecific confrontation between male rats and mice, is a relevant paradigm that can be used to understand the physiologic and behavioral adaptations to repeated stress. A typical social defeat paradigm evokes social confrontation between two animals belonging to the same species, in

Keywords: Sensory contact model; Psychosocial stress; Immune function; Winners and losers; Mice.

Abbreviations: EDTA, disodium salt of ethylene diamine tetracetic acid; WBC, white blood cells; ELISA, enzyme-linked immunosorbent assay.

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which the winner (dominant) and the loser (subordinate) animals can be identified at the end of the social interaction.[5] The chronic social conflicts or sensory contact model represents similar stressful situations that human beings encounter in everyday life. In this study, a developed animal model for chronic social stress in mice has been utilized. It is considered as a model of social defeat or subordination and dominance, and therefore may imitate situations occurring in humans.

Sensory contact model was originally used for studying the mechanisms of aggressive and submissive behaviors of male mice.[6] It was shown that repeated experience of victory or defeat in daily agonistic interactions leads to the formation of persistent opposing kinds of social behavior for the winners (aggressors) and the losers (victims of aggression). Relying on the emotional state (positive or negative), multiple neurochemical alterations in the synthesis, catabolism and receptors of key brain neurotransmitters can occur and are followed by behavioral and physiological changes in male mice.[7–9] Meanwhile, it has been shown that long exposure to social confrontations leads to psychoemotional disturbances, somatic disturbances and behavioral pathologies.[10]

The type and degree of pathology depend on both social behavior and the duration of agonistic interactions. Additionally, mice from different strains develop different pathologies, even though they share the same experience.[11] For example, in males of the C57BL/6J strain, a long experience of social defeat leads to the development of a mixed anxiety-depression state.[12,13] At the same time, the same stress caused by social confrontations in the defeated males of the CBA/Lac strain leads to the development of catalepsy.[7,14]

Therefore, the chronic social conflicts, from one perspective, are useful in inducing and investigating various psychoemotional and psychosomatic disturbances in animals.[15] On the other hand, it gives the opportunity of using animals with behavioral pathology as means to explore the action of novel and commonly used psychotropic drugs and to conduct their screening under simulated clinical conditions.[7] Consequently, the aim of the present work was to establish the use of a sensory contact model for studying the impact of psychosocial stress in mice. The study also aimed to investigate its influence on behavioral, neurochemical and immunological changes of both winners and losers.

Materials and methods

Animals

Adult male Swiss mice (25 ± 5 g) used in this study were obtained from the animal house of El Nile Co. for Pharmaceuticals (El Amyria, Egypt). The animals were kept together before the experiment in the animal house of Al-Azhar University for at least 1 week, for accommodation under suitable laboratory conditions at 25 ± 2 °C and on standard diet pellets with tap water ad libitum. In addition, the animals were not exposed to any stressful effects. The work was performed according to the ethical guidelines of Al-Azhar University (Faculty of Pharmacy), Egypt.

Experimental design

Forty adult male mice were divided into four groups, as follows: group 1, mice received normal saline (1 mL/kg, intraperitoneal) and were set in normal housing conditions to serve as the controls; group 2, mice received normal saline (1 mL/kg, intraperitoneal) and were caged individually for 5 days before sacrifice at the end of the experiment to abolish group effects and to serve as the control isolation group[16,17]; group 3, animals were subjected to sensory contact modeling for 21 days to produce the sensory winners; group 4, animals were subjected to sensory contact modeling for 21 days to produce sensory losers. After 21 days of sensory contact modeling, all mice were subjected to open-field test for behavioral evaluation.

At the end of the experiment, blood samples were withdrawn from the retroorbital plexus of mice from each group at day 22. The blood samples were collected from each animal into a clean, dry tube containing EDTA solution and used for evaluation of total and differential leukocyte count (neutrophils, lymphocytes, monocytes and eosinophils; as percentages). After that, the animals were sacrificed following the blood sample withdrawal and their whole brains were rapidly isolated and used for the preparation of 20% tissue homogenates in saline solution. The same volume of brain homogenate was used to investigate brain monoamines (dopamine, norepinephrine and serotonin).

Behavioral experiments

Open-field test

This is a general test for motor activity, excitability, emotionality and exploratory behavior in rodents. It is considered as one of the most important procedures in the majority of behavioral studies. The open-field test consists of a square wooden box (40 × 40 × 25 cm height),[18] with red walls and a white bottom that was divided into 16 equal squares (5 × 5 cm each) by using permanent paint.[19,20] The experiment was performed under white light, in a quiet room, between 1:00–3:00 pm to minimize the influence of possible circadian changes. All animals were taken to the test situation after removing food and water from the home cage 1 h before the experiment.

Each mouse was placed gently in the middle of the arena and videotaped for 5 m using a video camera (Supplementary video S1). The animal was then returned to the home cage. The open-field was thoroughly wiped using 10% isopropyl alcohol and dried before application of a new subject, in order to avoid possible biasing effects due to odor clues that may have remained from previous mice. The behavior of the experimental rat in the open-field test was continuously monitored during the 5 m observation period using coded symbols for the following parameters[21]: latency; time in s elapsed from placement of the animal at the middle of the arena until it makes the first move,[22] measured in seconds using a stopwatch; ambulation frequency, the number of squares the animal entered with all four paws,[20,23] which was scored as a total count during a 5-m period; rearing frequency, which was the number of times the animal stood on its hind limbs and stretched with and without forelimbs support, which was scored during a 5-m observation period; and, grooming time, which was calculated as time spent while the animal was scratching its face, licking its paws, fur or genitals.[24]

Biochemical parameters

Neurochemical parameters (dopamine, norepinephrine, serotonin)

Mice were sacrificed rapidly by decapitation, with minimum disturbance to avoid any changes in the concentrations of brain

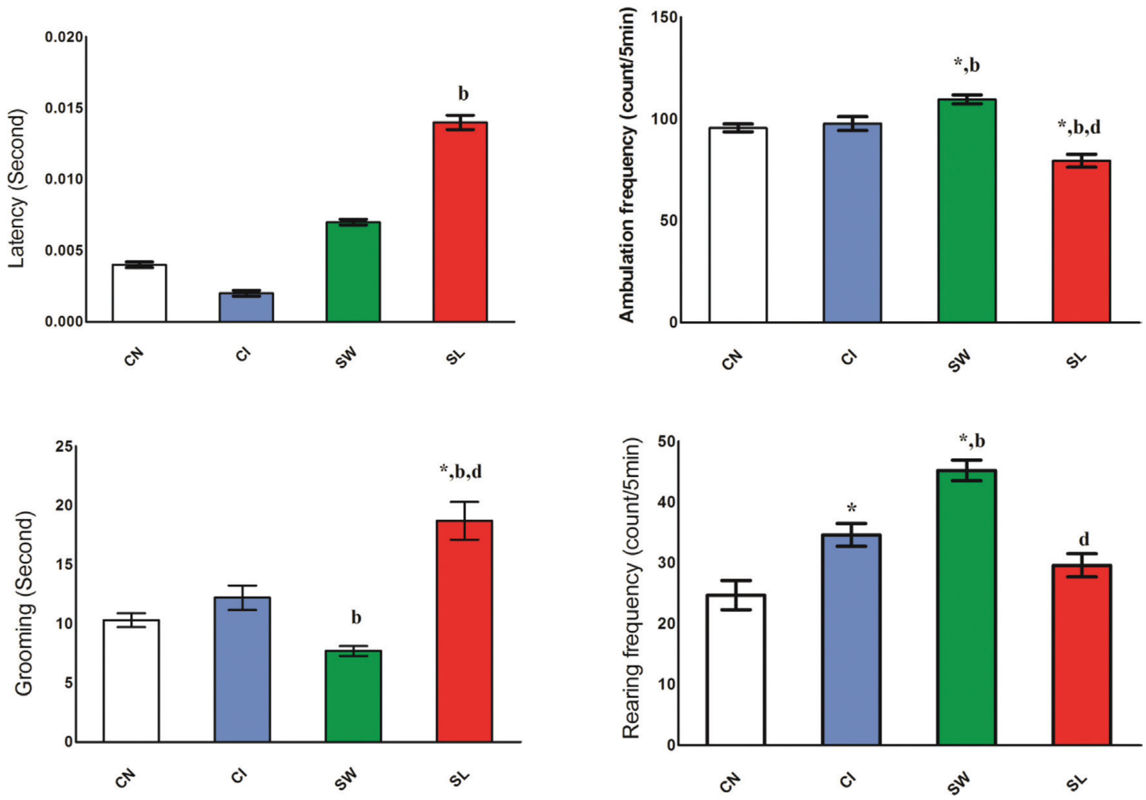

Fig. 1. Effects of sensory contact modeling on different behavioral parameters in the open-field test. Data are expressed as (mean ± standard error of the mean) (n = 10). CN: normal control mice, CI: control isolation mice, SW: sensory winners, SL: sensory losers. *, b, d are significantly different from CN, CI, SW, respectively at $p < 0.05$ using one-way analysis of variance followed by Tukey's multiple comparison test.

monoamines that may occur within a few m.[25] Determination of dopamine was assessed using commercial ELISA kit (Dopamine Research EIA; Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany).[26] Determination of norepinephrine was achieved using commercial ELISA kit (Noradrenaline Research EIA; Labor Diagnostika Nord GmbH & Co. KG).[26,27] Finally, determination of serotonin was carried out using the corresponding commercial ELISA kit from Labor Diagnostika Nord GmbH & Co. KG.[28]

Immunological evaluation

Assessment of total leukocyte count

A diluent (1% ammonium oxalate) was added to the well-mixed anticoagulated (EDTA) venous blood at a specific volume in the Unopette reservoir. The diluent lysed the erythrocytes but preserved the leukocytes and platelets. The diluted blood was then added to the hemocytometer chamber. Cells were allowed to settle for 10 m before the leukocytes were counted, which was carried out by counting in the four outside large squares of a counting chamber using light microscope under 10× magnification.[29,30]

The final cell count was reported as the number of white blood cells per microliter (WBC/µL) using the following formula:

$$\text{WBC}/\mu\text{L} = \frac{\text{Average of cells} \times \text{Correction for dilution}}{\text{No. of squares counted} \times \text{Volume of one square}}$$

Average of cells: The average of the total number of cells counted in the four large squares on both sides of the hemocytometer. Correction for dilution: The dilution factor (the reciprocal of the blood dilution). The dilution when using the white blood cell Unopettes was 1/100, so the dilution factor was 100. Number of squares counted: (4). Volume of one large square: 0.1 µL.

Assessment of differential leukocyte count

Anticoagulant blood (10 µL) was spread on a clean dry glass slide by the aid of a spreader slide with polished edges. Blood smears were air-dried and then flooded with Leishman's stain for 3 m, then gently washed with distilled water that was added slowly with a plastic Pasteur pipette. Slides were then left for 12 m and excess stain was washed off with slowly running tap water.[31] Finally, slides were held in a tilted position to facilitate drying and then examined under light microscope. One hundred leucocytes were examined and the percentage of the specified type of leucocytes (neutrophils, lymphocytes, monocytes and eosinophils) were recorded.[32]

Statistical analysis

Data are presented as mean ± standard error of the mean. Multiple comparisons were performed using one-way analysis of variance followed by Tukey's multiple comparison test as a post hoc test. All statistical analyses were performed and graphs made using GraphPad Prism (ISI®, USA) software (version 5).

Results

Performance in the open-field test

As illustrated in Figure 1 (panels i, ii, iii and iv), isolation did not significantly change the latency time, as compared to normal control mice. In contrast, the sensory contact modeling induced significant elevation in the latency time (to 700% in losers only, as compared to isolation group). Isolation did not significantly alter ambulation frequency, as compared to normal control mice. On the other hand, the sensory contact modeling induced significant elevation in the ambulation frequency in winners (to 114.64% and 112.2%, as compared to normal control mice and isolation groups respectively). However, losers showed induced significant reduction in ambulation frequency (to 83.2%, 81.4% and 72.53%, as compared to normal control mice, isolation and winners group, respectively).

Isolation induced significant elevation in rearing frequency (to 140.1%, as compared to normal control mice). Also, sensory contact modeling induced significant elevation in rearing frequency in winners (to 183% and 130.63%, as compared to normal control mice and isolation groups, respectively). Losers showed significant reduction in rearing frequency (reaching 65.5%, as compared to winners). Isolation did not significantly change the grooming time, as compared to normal control mice. On the other hand, sensory contact modeling induced significant reduction in grooming time in winners (to 63.11%, as compared to isolation group only). But, losers showed induced significant elevation in grooming time (to 181.6%, 153.3% and 242.9%, as compared to normal control mice, isolation and winner groups, respectively).

Neurochemical parameters (dopamine, norepinephrine and serotonin)

As illustrated in Figure 2 (panels i, ii and iii), isolation induced significant elevation in the brain dopamine content (to 156%, as compared to normal control mice). Also, sensory contact modeling induced significant elevation in the brain dopamine content in winner partners (to 239.6% and 153.6%, as compared to normal control mice and isolation groups, respectively). Additionally, loser partners showed induced significant elevation in the brain dopamine content (to 370.7%, 237.65% and 154.75%, as compared to normal control mice, isolation and winners groups, respectively).

Isolation induced significant elevation in the brain norepinephrine level (to 148.4%, as compared to normal control mice) and sensory contact modeling induced significant elevation in the brain norepinephrine level in winner partners (to 230% and 155%, as compared to normal control mice and isolation groups, respectively). Also, loser partners showed induced significant elevation in the brain norepinephrine level (to 342.02%, 230.5% and 148.7%, as compared to normal control mice, isolation and winners groups, respectively).

Isolation induced significant reduction in the brain serotonin level (to 76%, as compared to normal control mice) and sensory contact modeling induced significant reduction in the brain serotonin level in winner partners (to 56.5% and 74.32, as compared to normal control mice and isolation groups, respectively). Also, loser partners showed induced significant reduction in the brain serotonin level (to 38.18%, 50.26% and 67.62%, as compared to normal control mice, isolation and winners groups, respectively).

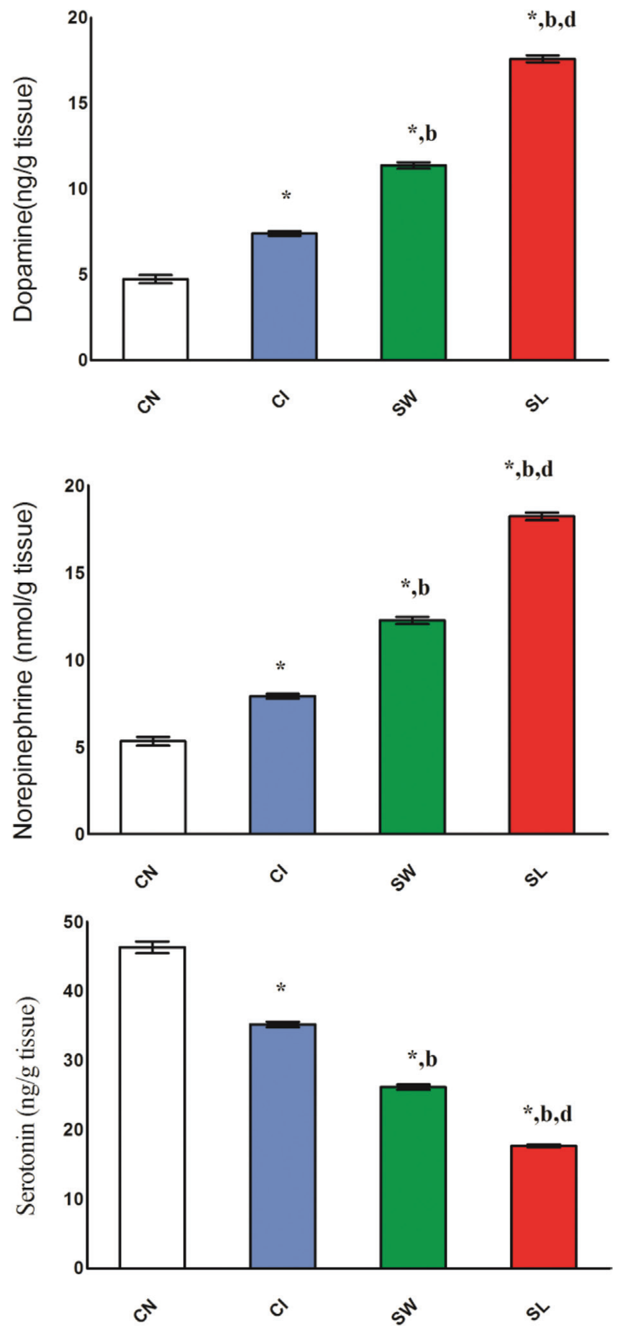

Fig. 2. Effects of sensory contact modeling on brain neurotransmitters. Data are expressed as (mean ± standard error of the mean) (n = 10). CN: normal control mice, CI: control isolation mice, SW: sensory winners, SL: sensory losers. *, b, d are significantly different from CN, CI, SW, respectively at $p < 0.05$ using one-way analysis of variance followed by Tukey's multiple comparison test.

Immunological parameters (total leukocyte count, neutrophils %, lymphocytes %, monocytes % and eosinophils %)

As illustrated in Figure 3 (panels i, ii, iii, iv and v), isolation induced significant elevation in total leukocyte count (to 142.7%, as compared to normal control mice) and sensory contact modeling

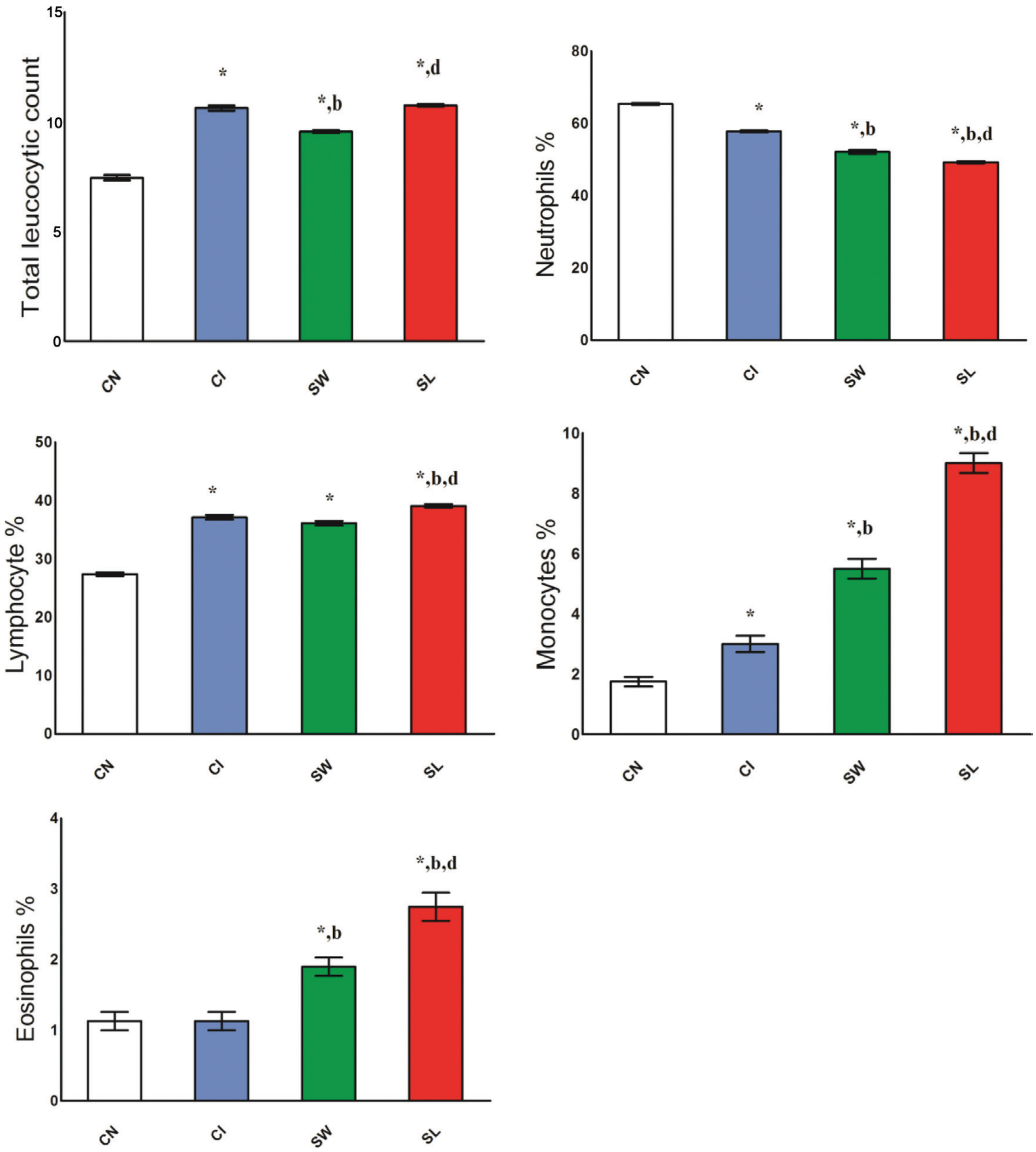

Fig. 3. Effects of sensory contact modeling on total and differential leukocyte count. Data are expressed as (mean ± standard error of the mean) (n = 10). CN: normal control mice, CI: control isolation mice, SW: sensory winners, SL: sensory losers. *, b, d are significantly different from CN, CI, SW, respectively at $p < 0.05$ using one-way analysis of variance followed by Tukey's multiple comparison test.

induced significant elevation in total leukocyte count in winner partners (to 128.32%, as compared to normal control mice), but significantly decreased total leukocyte count (to 90%, as compared to isolation group). Also, loser partners showed induced significant elevation in total leukocyte count (to 144.43% and 112.6%, as compared to normal control mice and winners groups, respectively).

On the other hand, isolation induced significant reduction in neutrophils percentage (to 88.38%, as compared to normal control mice) and sensory contact modeling induced significant reduction in neutrophils percentage in winner partners (to 79.7 and 90.2%, as compared to normal control mice and isolation groups, respectively). Also, loser partners showed induced significant reduction in neutrophils percentage (to 75.31%, 85.21% and 94.5%, as compared to normal control mice, isolation and winners groups, respectively).

However, isolation induced significant elevation in lymphocytes percentage (to 135.61%, as compared to normal control mice) and sensory contact modeling induced significant elevation in lymphocytes percentage in winner partners (to 132%, as compared to normal control mice group). Also, loser partners showed induced significant elevation in lymphocytes percentage (to 142.9%, 105.4% and 108.3%, as compared to normal control mice, isolation and winners groups, respectively).

Isolation induced significant elevation in monocytes percentage (to 171.43%, as compared to normal control mice) and sensory contact modeling induced significant elevation in monocytes percentage in winner partners (to 314.3% and 183.3%, as compared to normal control mice and isolation groups, respectively). Also, loser partners showed induced significant elevation in monocytes percentage (to 514.3%, 300% and 163.64%, as compared to normal control mice, isolation and winners groups, respectively).

Moreover, isolation did not significantly change eosinophils percentage compared to normal control mice, but sensory contact modeling induced significant elevation in eosinophils percentage in winner partners (to 168.1% and 168.1%, as compared to normal control mice and isolation groups, respectively). Also, loser partners showed induced significant elevation in eosinophils percentage (to 243.4%, 243.4% and 144.74%, as compared to normal control mice, isolation and winners groups, respectively).

Discussion

Social defeat is a very powerful stressor and can lead to a variety of behavioral effects, such as social withdrawal (reduced interactions between two animals belonging to the same species), lethargy (reduced locomotor activity), reduced exploratory behavior (in both open-field test and novel objects), anhedonia (reduced reward-related behaviors), and decreased sociosexual behaviors (including decreased attempts to mate and copulate after defeat), as well as augmented anxiety.[33–35]

In the current study, winner partners showed an increase in motor activity, which was manifested by a significant increase in ambulation and rearing frequencies in the open-field test compared to the control and isolation groups, and was associated with a significant decrease in grooming from isolation group. These findings are in agreement with Sandnabba who mentioned that the increase in aggressive behavior in winner partners was accompanied by an increase in motor activity.[36] In addition, some studies have recognized an increase in movement in open-field test and forced swimming tests in conditions of repeated experience of aggression.[37]

Loser partners showed a decrease in motor activity, which was manifested by a significant decrease in ambulation frequency in the open-field test compared to the control, isolation and winners groups, and a significant decrease in rearing frequency compared to winner group, and this was associated with a significant increase in grooming compared to the control, isolation and winners groups. These findings are in agreement with Bjorkqvist and Berry *et al*.,[38,39] who showed that losers had induced decline in locomotor activity and exploring behavior. The losers developed a severe behavioral deficit after experiencing social defeat.[40,41] Previous studies have reported that social conflict has also been found to cause permanent behavioral changes in rodents, including the development of anxiety-like behaviors.[42]

Other studies estimating the effects of chronic psychosocial stress on behavioral outcomes have employed the elevated plus-maze test, in which a decrease in social interest and lack of social preference has been reported to reflect enhanced depression-related behavior.[43,44] However, decreased importance in the exploration of two animals belonging to the same species may be clarified as the display of social avoidance or social anxiety.[45,46] Furthermore, Kudryavtseva and Avgustinovich demonstrated that social conflicts in daily agonistic confrontations have led to disturbances in social life and also to changes in the loser's behavior in different situations.[12] The results obtained demonstrated a significant decrease in the ambulation and exploratory activity of depressive mice in open-field and exploratory activity tests.

These results may be a consequence of a decrease in exploration in new conditions as a result of fear, and the decrease in ambulation may testify, possibly, to losers' decreased energy as a consequence of developing pathology. On the contrary to the present study, a previous study showed that experience of aggression in male C57BL/6J mice led to the development of anxiety, evaluated using a variety of tests. The development of anxiety in aggressive males may be the result of prolonged social stress induced by involvement in agonistic confrontations.[47]

A possible explanation of this disagreement may be attributed to species differences; sex differences may also be a factor. However, it can be suggested that the development of anxiety in aggressive males occurs as a result of neurochemical changes arising in the brain under the influence of repeated experience of aggression, the matter which causes imbalance between the neurotransmitter systems involved in forming the aggressive type of behavior.[37,48] Finally, psychological studies have noticed that aggression and anxiety are correlated by an orthogonal relationship; some subjects have high aggression and high anxiety, some have high aggression and low anxiety, some have low aggression and low anxiety, and finally some have low aggression and high anxiety.[49,50]

In the present study, it was observed that repeated experience of aggression in winners is accompanied by a significant increase in brain dopamine and norepinephrine levels from the control and isolation groups. The loser partners showed an increase in brain dopamine and norepinephrine levels from the control, isolation and winners groups. These findings are in agreement with Bjorkqvist,[51] who found that epinephrine and norepinephrine were increased in losers. Also, Alekseenko *et al*.[52] and Avgustinovich *et al*.[53] showed that blockade of D2 receptors by sulpiride modifies the behavior of the losers near the partition. Previous studies have demonstrated that stress activates the brain's noradrenergic system, thus increasing concentrations of the transmitter itself and accelerating its turnover, respectively. Enhanced norepinephrine release in projection areas of the locus coeruleus correlates with high expression of tyrosine hydroxylase, the rate-limiting enzyme of the norepinephrine biosynthesis pathway.

Stress-induced elevation of monoamine concentrations are, in part, also due to decreased degradation of the neurotransmitters related to diminished activity of monoamine oxidases.[54,55] Also, Fuchs and Flugge noticed an activation of the mesolimbic dopaminergic system by acute stress in mice where restraint stress or foot shock raise dopamine release in the mesolimbic system.[55] In contrast, longer or repeated exposure to stress has been assumed to decrease dopamine release. Accordingly, changes in dopamine receptor binding have been noticed in the hippocampus during a prolonged period of social stress. The majority of noradrenergic neurons are found in the locus coeruleus. Altered noradrenergic signaling is linked to anxiety disorders. Sustained activation of locus coeruleus results in manifestation of anxiety symptoms. Stress-induced release of norepinephrine facilitates a number of anxiety-like behavioral responses, including stress-induced reduction of open arm exploration on the elevated plus-maze and stress-induced reduction of social interaction.[56–58]

The present study disagrees with the work of Kudryavtseva and Kudryavtseva *et al*.[10,59] These researchers observed that there was an activation of the dopaminergic and noradrenergic systems in winners, with repeated experience of aggression. In contrast to the present results, repeated experience of social defeats led to the attenuation of the activity of serotonin, norepinephrine and dopamine,[10,13] which indicates that losers are under physiological stress. Other pharmacological studies showed that dopamine receptors are involved in the neural mechanisms associated with the development of aggressive behavior in male mice under the influence of repeated experience of aggression.[60,61] Previous studies demonstrated that dopamine plays a major role in modulation of aggressive behaviors.

In animal studies, hyperactivity in the dopamine system is correlated with increases in impulsive aggression.[62,63] Studies on aggressive behaviors in rodents demonstrated that elevated dopamine levels have been continuously noticed before, during and following aggressive fights.[64] In humans, the dopaminergic system has been associated with the recognition and experience of aggression. After administering a dopamine D_2 receptor antagonist, sulpiride, subjects revealed an impaired ability to recognize angry facial expressions.[65] Also, there is evidence that impulsive behavior may be enhanced by elevated dopaminergic function.[66] In addition, dopaminergic hyperfunction is connected with impulsivity and emotional dysregulation in patients with borderline personality disorders.[67] Finally, dopamine levels manipulated pharmacologically have been shown to increase or decrease aggressive behavior.[63,68]

In the present study, it was observed that repeated experience of aggression in winners is accompanied by a significant decrease in brain serotonin level in the control and isolation groups. The loser partners showed a severe decrease in brain serotonin level compared to control, isolation and winners groups. These findings are in agreement with Avgustinovich *et al*.,[69] who reported that repeated experience of defeats in 10 daily agonistic confrontations produced pronounced anxiety in C57BL/6J male mice (losers), as evaluated by some plus-maze parameters, and decreased communicative behavior, as observed in the partition test. Tryptophan hydroxylase activity and the levels of serotonin and 5-hydroxyindole acetic acid in the midbrain, hypothalamus, amygdala, hippocampus and striatum have been calculated and analyzed in anxious losers.

These data suggested that pronounced anxiety developing in losers was accompanied by changes of serotonin metabolism in the various brain areas differently. A lower 5-hydroxyindole acetic acid level and 5-hydroxyindole acetic acid/serotonin ratio in the hippocampus, as well as lower 5-hydroxyindole acetic acid level and decreased tryptophan hydroxylase activity in the amygdala, existed in the anxious losers in comparison with the control (5 days of individual housing). Elevated 5-hydroxyindole acetic acid level and 5-hydroxyindole acetic acid/serotonin ratio were observed in the midbrain of the losers. The increased tryptophan hydroxylase activity was found in the hypothalamus of the losers. It was noticed that the development of pronounced anxiety in the losers due to the repeated social confrontations was accompanied by hypoactivity of the serotonergic system in the amygdala and hippocampus.[69] Based on preclinical and clinical evidence, the brain serotonergic system plays a key role in the expression of anxiety and depression. There is great evidence that the serotonin 1A agonists have antidepressant effects, both in mice and rats. Interest is growing in the serotonin 1A receptors as primary targets for anxiotropic and antidepressant drugs.[53,70]

Previous studies showed that chronic social stress or repeated intermale confrontations cause depressive-like behaviors in the losers, and chronic treatment with either ipsapirone or buspirone does not improve their status.[53] In addition, Mann *et al*.[71] reported that serotonin hypofunction appears to be related to human depression. Markers for the serotonergic system were decreased in the brain of depressed suicide victims. Also, serotonergic neurons in the brain stem innervate nearly every part of the brain. In concurrence with the wide expansion of the serotonergic fibers, the system is involved in a large number of brain functions, including emotional processing. The discharge patterns of neurons in the dorsal raphe nucleus and the release of serotonin changes across the sleep-wake cycle, and when an animal becomes behaviorally active, serotonin release increases.

A previous study also, found that stress alters serotonin 1A receptors in target regions of the dorsal raphe nucleus.[55] Also, Avgustinovich *et al*.[13] reported that hypofunction of the serotonergic system may occur at the stage of pronounced depression in animals. Similar processes have a place in brain dopaminergic systems. It has been revealed that dynamic changes of brain monoaminergic activities accompany the development of anxious depression in animals. Various parameters of monoaminergic systems are differently changed, depending on brain area, mediator system and stage of disorder. The current data do not allow making obvious conclusions about the regulation of the serotonergic system because the data and hypotheses are often contradictory. Some authors connect the mechanisms of depression to a functional decline of brain serotonin, whereas other authors connect it to a hypersensitivity of the serotonin receptors or a hyperfunction of the serotonergic system. Similarly, disturbances in the serotonergic-catecholaminergic balance are expected to be a cause of the depressive illness.[12,72,73]

Analysis of the present data showed that winner partners had a significant increase in total leukocyte count compared to the controls. The significant increase in lymphocytes in winners may contribute to the significantly higher total leukocyte count. Such a finding has also been observed in a previous study.[18] Loser partners showed increased total leukocyte count compared to the control and winner groups. Also, loser partners showed increased lymphocytes from the control, isolation and winners groups. In contrast to the present results, other authors have shown a decrease in the percentage composition of lymphocytes and an increase in the composition of segmented neutrophils in the blood of aggressors and victims compared with controls.[74] In addition, various rodent models have suggested that social stress can encourage changes in the immune system, such as altered leukocyte subset populations.[75,76]

Winner partners had significantly decreased neutrophils' percentage, as compared to the control and isolation groups, respectively. Loser partners had significantly decreased neutrophils percentage, as compared to the control, isolation and winners groups, respectively, and winner partners showed a significant increase in neutrophils percentage from losers. This may be due to high aggression in winners, which is more than losers and which stimulates blastogenesis and increases cellular immunity. These findings are in agreement with those of Line *et al*.[77] who reported that repeated aggression in winners stimulates blastogenesis and increases cellular immunity, while low aggression in losers leads to reduction of blastogenesis and cellular immunity. Investigations of primary immune response in losers showed a decrease in immune functions.[12] In contrast to this, the present work found that winner partners revealed a significant decrease in total leukocyte count, lymphocyte, monocytes and eosinophils, and showed a significant increase in neutrophils percentage from loser partners.

The National Institute of Health states that stress can cause the number of white blood cells to rise. This occurs because the immune system is designed to manage or prevent disease. Social stress in mice can result in fighting-induced wounds. About 90–95% of the loser mice experience trauma during social conflicts. Trauma causes activation of nearly all components of the immune system. It activates the neuroendocrine system and local tissue destruction, and accumulation of toxic byproducts of metabolic respiration leads to release of mediators. Extensive tissue injury may result in spillover of these mediators into the peripheral blood stream to further maintain and enhance the proinflammatory response. Hormones like ACTH, corticosteroids and catecholamines, as well as cytokines, chemokines and alarmins, play important roles in the initiation and persistence of the proinflammatory response after severe injury.[78,79]

Defeated mice exhibited an elevated level of serum corticosterone. Many of the immunological reactions in submissive males may be mediated by the action of glucocorticoids. Glucocorticoids decrease the ability of lymphocytes to adhere to the endothelium and impact the migration of various lymphocyte subsets from the blood into the lymph nodes or bone marrow.[80,81] This could result in differential accumulation of T and B cells in the blood, as seen in submissive males. Second, glucocorticoids have been shown to increase release of neutrophils from bone marrow stores,[82] but the apparent neutropenia in losers may be due to increased margination of neutrophils from the circulating pool to the marginal blood or tissue pools.[83,84]

Repeated exposure to social stress induced a state of glucocorticoids resistance in peripheral immune cells. Glucocorticoids resistance developed in losers and was linked to the presence of injuries due to fighting, but not to alterations in systemic levels of corticosterone. Since the loser is associated with increased risk for injuries due to fighting, it may be that the development of glucocorticoids resistance is an adaptive mechanism that allows the inflammatory component of wound healing to occur in the presence of high levels of corticosterone.[85–88] So, the phenomenon of glucocorticoids resistance was most obvious in mice that were subordinate and received severe bite wounds.[85,87] In addition, it is well known that depression in humans is accompanied by different somatic symptoms (*e.g.*, weight loss and disturbances in gastrointestinal functions). So, neutropenia may be due to nutritional deficiency, such as vitamin B_{12} and folate (folic acid) deficiency.[53,84]

In contrast to the present results, previous results of the immunological effects of sensory contact modeling showed that winners have stronger immune-stimulating response than losers.[89,90] Traditionally, both stress and depression have been associated with impaired immune function and increased susceptibility to infectious and neoplastic disease. Despite the initial finding that immunosuppression occurs in depression, some studies have shown that immune activation could also be present and might even play a role in the onset of depressive symptoms.[72,91] Psychological stress is known to decrease immune function and increase susceptibility to infections and cancer. Paradoxically, stress is also known to aggravate some allergic, autoimmune and inflammatory diseases, which suggests that stress may augment immune function under certain conditions.

It has been reported that chronic stress suppresses or dysregulates immune function, and acute stress often has immunoenhancing effects. One of the most under-respected effects of stress on the immune system is its ability to induce significant changes in leukocyte distribution in the body. Importantly, these changes have significant effects on immune function in different body compartments that are either enriched or depleted of leukocytes during stress.[92,93]

It is worthy to note that two "pathways" by which the immune system is modulated by psychological stress include the hypothalamic–pituitary–adrenal axis and the sympathetic-adrenal medullary axis. In addition, there is direct innervation of primary and secondary lymphoid tissues by the autonomic nervous system. These "pathways" function by producing biological mediators that interact with and influence cellular components of the immune system.[94] Quana *et al*.[95] reported that enormous traumatic stress and depression may result in hyperinflammatory states. These phenomena suggest that under certain conditions, stress or the release of stress hormones may not be antiinflammatory, and the development of glucocorticoids resistance has been recommended as one of the mechanisms by which a hyperinflammatory state may be induced under stress.

Recommendations and future directions

Social stress represents a risk factor for most psychological disorders; it is highly correlated with immune function and may also affect other organs, especially heart. Consequently, it is recommended to use a sensory contact model for studying the potential role of social stress on different body organs, either in the winners or in the losers. Sensory contact modeling can be also used to explore the efficacy of many protective agents against stress-induced degenerations, especially the efficacy of anxiolytics and natural antioxidants. Additionally, it can also be used to study the interaction between social stress and side effects of drugs, especially when the drug is widely used and clinically effective, but its use is limited due to the adverse effects which may be deteriorated by stress.

Conclusions

Social stress induced by using sensory contact modeling represents a risk factor for many psychological disorders and leads to severe depression as well as anxiety-related behaviors. It also reduces locomotor activity and exploratory behavior; losers were more depressed, while winners were more aggressive. Conversely, it enhances the immune function in both winner and losers. The enhancement of the immune function was more pronounced in losers than winners. This effect may be attributed to the threatened sensation induced by stress, especially in losers, as well as to the trauma that occurs in losers during exposure to sensory contact modeling.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Developing the research idea (AAA), supervising the experiments execution (AAA), supervising the data analysis (AAA, HIA, BMB), revising the manuscript (AAA, HIA, BMB), designing the experiments (HIA, BMB), supervising the experiment execution (HIA, BMB), performing the experiments (HAE), collecting the data (HAE), analyzing the data (HAE), writing the manuscript (HAE).

Supporting information

Supplementary material for this article is available at https://doi.org/10.14218/JERP.2017.00017.

Video S1. Video of the behavior tests.

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