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EFFECT OF DIAZEPAM TREATMENT ON THE COMPETITIVE BEHAVIOR OF MALE RATS AND ASSESSMENT OF ANXIETY IN COMPETING RATS

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ABSTRACT

The current study was carried out to investigate the effect of diazepam treatment on the competitive behavior of male rats and assess the level of anxiety in the competing rats. Forty-four food-deprived rats were individually trained to consume a 50g regular food pellet whose signaled delivery at 30 seconds intervals. The subjects were housed as fixed dyads throughout the study to enhance the establishment of stable dominance hierarchy in the dyads. According to the results of the competition sessions, the twenty-two dyads were classified into 12 stable dyads and 10 unstable dyads. One half of each class of dyads was assigned for the anxiety study, while the other half was specified for the competition study. Winners and losers were treated with diazepam, 0.5mg/kg, i.p, 30min before testing. The anxiometer, LE 3206 control unit was used to assess anxiety level in the animals. The total trial length time was 5 minutes. Diazepam treatment of both stable winners and losers has disrupted the social hierarchy; treatment with diazepam enhanced the competitive behavior of few inhibited animals. In contrast, diazepam treatment could establish a stable dominance ranking in the previously 10 unstable dyads of rats. The Vogel conflict test showed that both winners and losers in different dyads had experienced different levels of anxiety. Treatment with diazepam could enhance the behavior of inhibited animals and it significantly increased the number of shocks received by the stable losers. The anxiolytic effect of diazepam was evident in the unstable dyads. It is concluded that social housing of rats could produce a dominance hierarchy; this model could induce a state of anxiety in these animals; treatment with an anxiolytic agent could improve their state of anxiety and enhance their competitive behavior.

INTRODUCTION

In human societies, interpersonal relationships constitute the main source of adverse and chronic stressful situations[1]. The social stress generated in these relationships is considered one of the main etiological factors in the development of different emotional disorders, including anxiety, depression[2] and substance abuse[3]. Thus, long-term relations of dominance-subordination appear to provide a suitable model for studying the behavioral changes associated with chronic stress[4] and their relationship with the development of psychopathologies. Animal research has revealed that subordinate animals may experience a high level of nonspecific threat coupled with a real possibility of physical attack[5]. These animals experience profound changes in their physiological and behavioral mechanisms[6].

Since the majority of stressful stimuli, which produce psychopathologies in human, are of social nature, the study of the consequences of social stress in experimental animal models is considered to be of great interest[7]. A number of different animal models for psychopathologies in human have been developed on the basis of stressful social stimuli, and have been found to have both face and predictive validity[8]. One of these psychosocial stress models is based on the resident-intruder paradigm, developed initially by Miczek[9]. Subsequently, numerous variations have been developed on the basis of this model. These include evaluation of spontaneous fighting among individual rats in social groups[10], competitive behavior for a sexually receptive female[11], or competitive behavior of animals for palatable food[12]. In this model, dominance and subordination are defined in competition trials that measure how successful food-deprived rats at accessing food at the expense of the opponent[13].

The present study was carried out to investigate the effect of diazepam on the competitive behavior of rats and study the possible relationship between the levels of anxiety and the competitive performance in these animals.

MATERIAL AND METHODS

Forty four male rats, obtained from the National Institute of Drug Control and research (Cairo, Egypt), with initial body weight 120±30 g were used in the present study. Animals were individually housed in stainless steel cages measured 35X25X20cm. Animals were kept at 25±3°C, with illumination on a 12/12 hr light-dark cycle throughout the study. Food and tap water were available ad libitum for one-week accommodation period. Tap water was also available ad libitum during the remainder of the study. However, during that time, the animals were food-deprived to enhance competition for food. Food availability was restricted to a daily session of feeding for 2 hrs (3:00-5:00p.m) after the experimental sessions for that day were concluded. The body weight of the animals has increased by 10-30g at the end of the study.

Food-deprived rats were individually trained to consume a 50g regular food pellet whose signaled delivery at 30 seconds intervals. Food pellets were delivered through a glass tube, 20cm long and 1cm diameter, into a food pot in the home cage. Food pellet delivery occurred manually immediately after the ringing of a stimulus bell. Ten pellets were delivered per session. Rats were trained daily, one session per day for 6 days.

Following completion of training, subjects were assigned to dyads (two rats per cage) based on similarity in the body weight. Each rat was coded with specific color (red or blue) using food coloring applied to the tail (twice a week) in order to distinguish each rat within its specific dyad. Following formation of 22 dyads, two feeders were supplied to the cage during the two-hour feeding session to enhance food availability to all subjects. Excess food was available during the feeding session. The subjects were housed as fixed dyads throughout the study to enhance the establishment of stable dominance hierarchy in the

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dyads. After a sufficient time has elapsed (one week) since dyads formation, dominance ranking was assessed.

Competition test sessions were conducted daily (10:00a.m-3:00p.m) in the home cage of the animal dyads. The food-deprived animals competed for the delivered food pellets. Competition was scored by direct observation. The animal got a score of one point for each ingested food pellet. The competition score for each animal /session is determined by adding up the number of ingested pellets per session. A rat was ranked as a winner or loser in a specific session based on its composite score within its specific dyad in that session[13]. A final rank order of animals was determined based on the average score of six consecutive daily sessions. A stable dominance ranking was achieved when subjects maintained the same rank position within a given dyad for four or more consecutive sessions. Rats in a given unstable dyads could not maintain the same rank order for more than three consecutive daily sessions.

Anxiety test: The Vogel conflict test of anxiety:

Vogel et al.[14] developed a conflict procedure - the Vogel conflict test- in which male rats were water-deprived for 48 hours and, during a test session of 3 min, drinking was punished by a mild but aversive electric shock delivered via the spout of the bottle every 20 licks. Accordingly, a specific, drug-induced increase in the number of shocks taken (equivalent to water drunk) was considered to reflect anxiolytic properties of a drug treatment; a decrease in the number of shocks taken by the animal reflects a state of anxiety.

Relatively few studies have modified the parameters of the Vogel conflict test in order to render it more sensitive to anxiogenic agents: this is important since, in particular where novel mechanisms of action are under exploration and drug actions are not known, it is advantageous that both increases and decreases in anxiety can be revealed.

The Vogel conflict test has proven of importance in the characterization of diverse classes of anxiolytics, and there remains considerable scope for its continued and improved application in the identification of novel agents and in the exploration of their mechanism(s) of action. The robust action of benzodiazepines in the Vogel conflict test parallel their clinical efficacy in patients; such observations support the notion that positive results in the Vogel conflict test are of clinical relevance. Common with other conflict models responsive to benzodiazepines, the Vogel conflict test is of rather broad significance to clinical anxiety[15]. Further, the Vogel conflict test appears to be of particular relevance to generalized anxiety disorders[16].

The anxiometer, LE 3206 control unit:

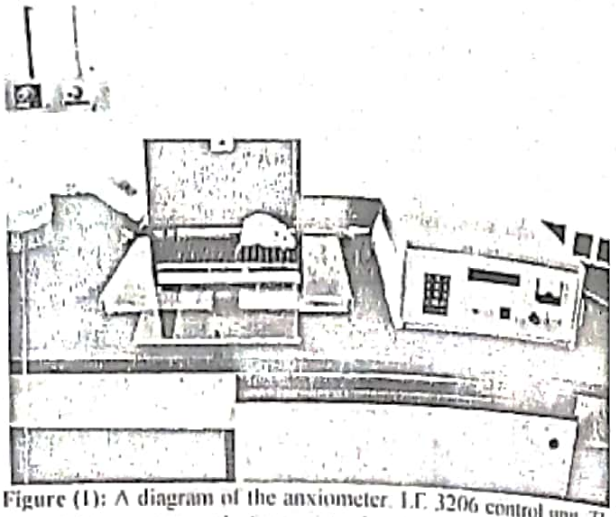

Figure (1): A diagram of the anxiometer, LE 3206 control unit. The anxiometer is composed of a testing chamber and a shock generator device

Experimental procedures for testing anxiety:

The shock parameters were set, shock length was 1 second, and shock intensity was 0.3mAmp. The licks/shock ratio (LSR) was fixed at 20-licks/1-shock; the total trial length (TTL) time was 5 minutes. A shock was supplied each time the LSR is completed; the TTL time started only after the first completion of the LSR. The trial run until the TTL time was completed.

Study design:

According to the results of the competition sessions, the twenty-two dyads were classified into 12 stable dyads and 10 unstable dyads. One half of each class of dyads was assigned for the anxiety study, while the other half was specified for the competition study. The study design and treatment regimens are summarized in table 1.

Table (1): A summary of the study design

22 Dyads			
10 Unstable Dyads		12 Stable Dyads	
5 Dyads for competition test	5 Dyads for anxiety test	6 Dyads for competition test	6 Dyads for anxiety test
Winners and losers were treated with diazepam*. 0.5mg/kg, i.p. 30min before testing	Winners and losers were treated with diazepam. 0.5mg/kg, i.p. 30min before testing	Winners and losers were treated with diazepam. 0.5mg/kg, i.p. 30min before testing	Winners and losers were treated with diazepam 0.5mg/kg, i.p. 30min before testing

*Diazepam (Valpam ampoule, Amoun Co., Egypt)

The animals of both the 5 unstable dyads and 6 stable dyads, which were subjected to anxiety study, were also water-deprived, therefore, both food and water were available for these animals for 2 hours a day (feeding time).

Statistical analysis:

Values are expressed as mean ± SEM for animals in each experimental group. Statistical analysis of the results was carried out by means of unpaired and paired t-test for evaluation of the competition study results and the anxiety results respectively. The level of significance was set at $p < 0.05$. Statistical tests were performed with Microsoft Office Excel 2003, Microsoft, USA.

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RESULTS

In the current study, the continued housing of animals in dyads has produced a stable dominance hierarchy in 12 dyads of rats. The competition study has revealed a significant difference in the average competition score between winners and losers (Stable winners, 8.1±0.4 vs. Stable losers, 1.9±0.6, $p< 0.05$, figure 2). In contrast, there was no significant difference between the average competition score of winners and losers of ten unstable dyads of rats (figure 2).

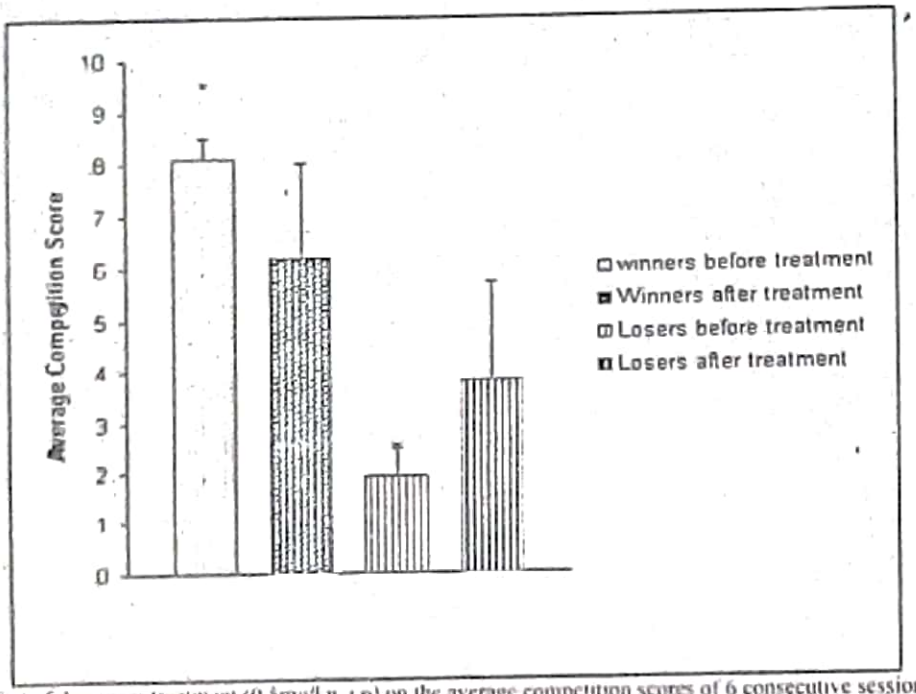

Figure (2): Effect of diazepam treatment (0.5mg/kg, i.p) on the average competition scores of 6 consecutive sessions of winners and losers across 6 pairs of stable dyads of rats

*Winners are significantly different from corresponding losers at $p<0.5$

Diazepam treatment of both stable winners and losers has disrupted the social hierarchy; treatment with diazepam enhanced the competitive behavior of few inhibited animals; two previous losers became winners after treatment in 4 consecutive competition sessions out of 6.

In contrast, diazepam treatment could establish a stable dominance ranking in the previously 10 unstable dyads of rats. Figure 3 shows that there is a significant difference between the average competition score of winners and losers of these dyads after treatment with diazepam (Unstable winners after treatment, 7.9±1.3 vs. Unstable losers after treatment, 2.1±0.7, $p<0.05$). Three unstable winners became winners in 4 out of 6 consecutive sessions of competition

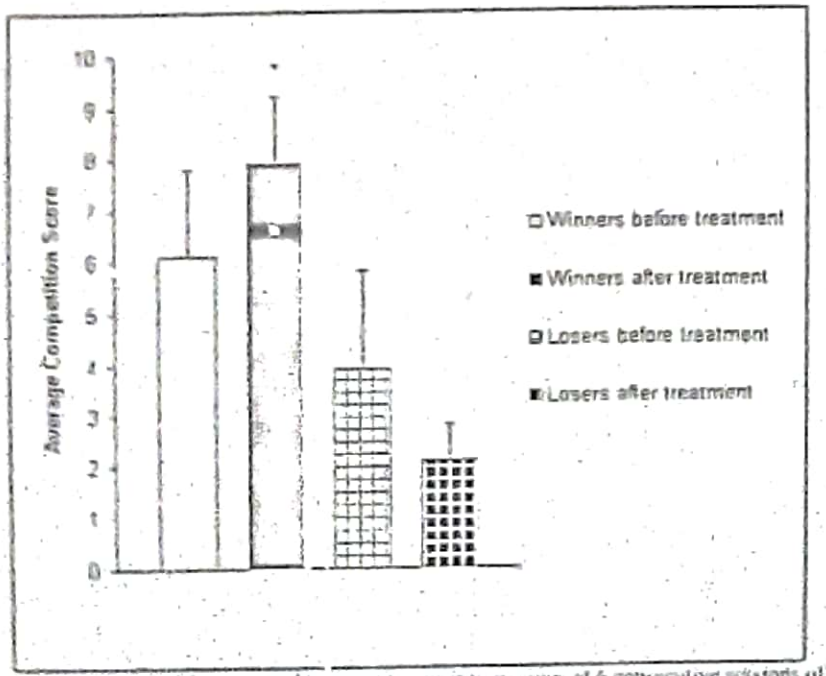

Figure (3): Effect of diazepam treatment (0.5mg/kg, i.p) on the average competition scores of 6 consecutive sessions of winners and losers across 5 pairs of unstable dyads of rats

* Winners are significantly different from corresponding losers at $p< 0.5$

The Vogel conflict test showed that both winners and losers in different dyads had experienced different levels of anxiety. Figure 4 shows that stable winners could receive a higher number of shocks as compared to the stable losers; the later were more inhibited (Stable winners, 24±3.2 shocks vs. Stable losers, 8±2.1 shocks; $p < 0.05$, figure 4), which was not evident in the unstable dyads (figure 4). In contrast, no significant difference was observed between the unstable winners and losers (figure 4); both were relatively inhibited.

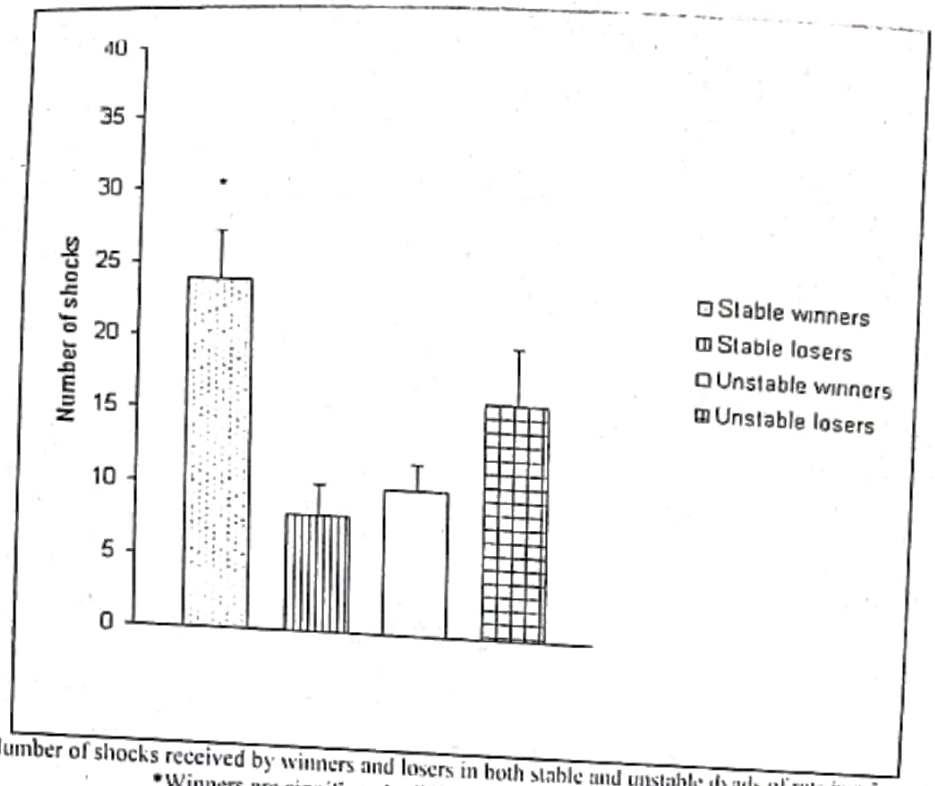

Figure (4): Number of shocks received by winners and losers in both stable and unstable dyads of rats in a 5-min anxiety test
*Winners are significantly different corresponding losers at $p < 0.5$

Treatment with diazepam could enhance the behavior of inhibited animals and it significantly increased the number of shocks received by the stable losers (Stable losers before treatment, 8±2.1 shocks vs. Stable losers after treatment, 26±2.8 shocks, $p < 0.05$, figure 5). No significant change was noted in the status of stable winners.

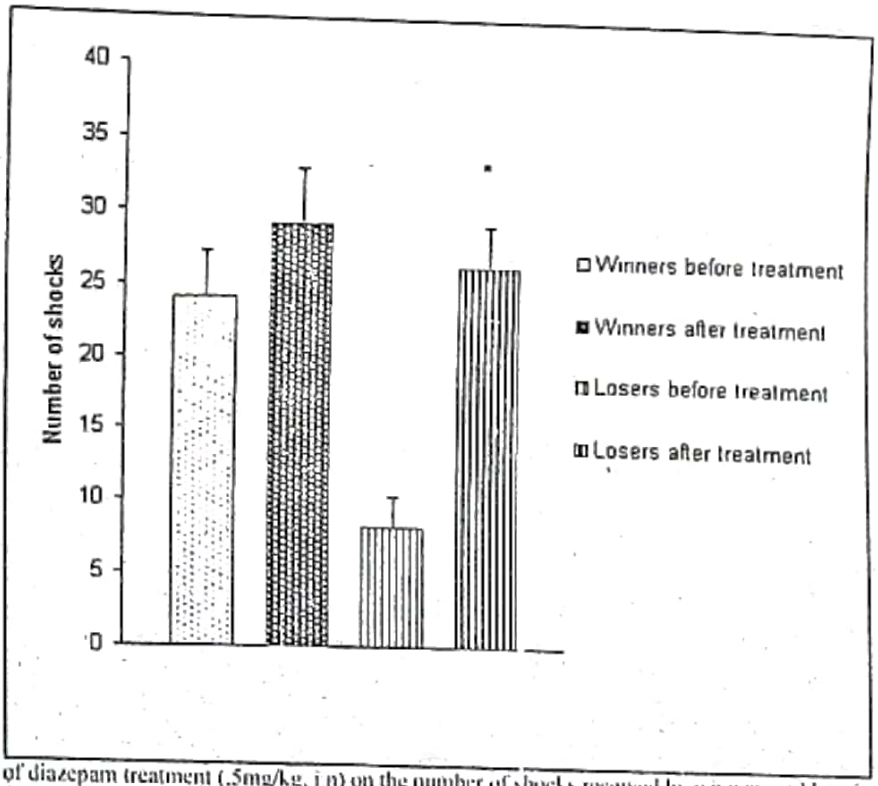

Figure (5): Effect of diazepam treatment (.5mg/kg, i.p) on the number of shocks received by winners and losers of 6 stable dyads of rats in a 5-min anxiety session

*Significant paired t-test at $p<0.5$

The anxiolytic effect of diazepam was evident in the unstable winners (Unstable winners before treatment, 10±1.7 shocks vs. Unstable winners after treatment, 23±2 shocks, $p< 0.05$, figure 6). A lower effect was demonstrated by the unstable losers (Unstable losers before treatment, 16±3.9 shocks vs. Unstable losers after treatment, 21±3.4 shocks, figure 6).

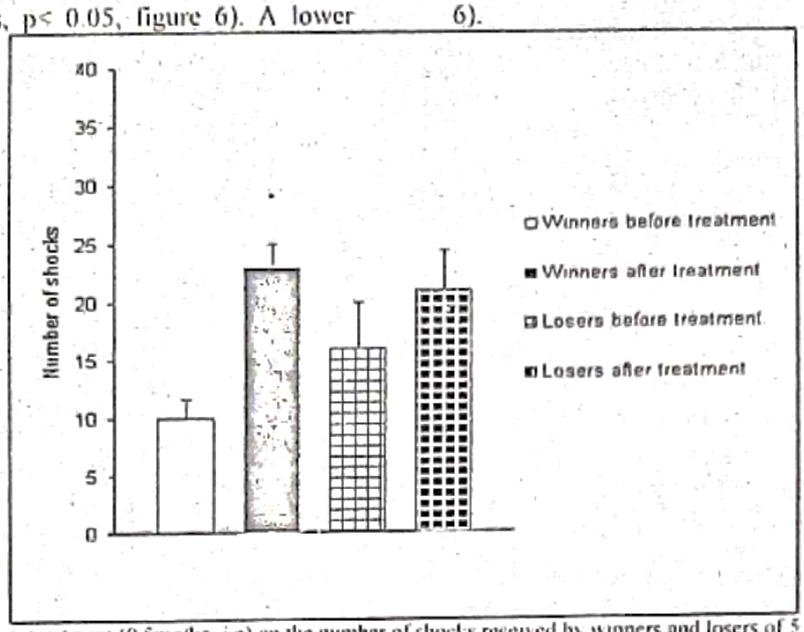

Figure (6): Effect of diazepam treatment (0.5mg/kg, i.p) on the number of shocks received by winners and losers of 5 unstable dyads of rats in a 5-min anxiety session

*Significant paired t-test at $p<0.5$

DISCUSSION

In the present model, the social housing of rats could produce dominant and subordinate animals in their specific dyads in a competition setting.. Dominant (winners) and subordinate (losers) animals experienced different levels of anxiety in the current Vogel conflict test.

It has been found that repeated experiences of social defeat or subordination is a psychosocial factor that provokes physiological and behavioral alterations in rodents that are related to neuro-endocrine changes coupled to a negative emotional state typical of anxious/depressive symptoms in human[17] and are sensitive to antidepressants treatments[18]. Further, many studies have shown that dominant individuals are also subject to the negative effects of chronic social stress[19]. Nevertheless, dominant individuals seem to be able to habituate themselves to the situation, while subordinates continue to show severe effects of exposure to stress for prolonged periods[20].

In agreement with the previous findings, the current study indicated that losers suffered from a higher level of anxiety as reflected by the lower number of shocks received by these animals; this anxiety was relieved by diazepam treatment.

Among the various behavioral changes observed in animals of social defeat, which are considered behavioral signs of depression, we can highlight hypophagia[21] and the alteration of circadian rhythms and the sleep-awake cycle[22]. A decrease in exploratory activity has also been observed within the subject's own cage, in open field test and in the activity box, and an increase in time spent immobile[23].

It is well known that depression is often accompanied by an anxious pathology, and indeed, many symptoms of depression and anxiety overlap and the comorbility between depression and anxiety disorders is more a rule than an exception. Anxiety is a prominent and prevalent symptom of depression[24]. Various studies have found that an increase in anxiety in defeated mice, manifested through a decrease in losers interaction behavior towards other conspecifics. Repeated defeat diminishes the total amount of time spent by mice near the barrier that separates them from their dominant counterpart[25]. This decrease in social interaction between male rodents in the laboratory has been related to a decrease in social communication, which may be caused by the development of anxiety and depression[26]. Studies using anxiety tests demonstrated that social defeat has been consistently related to an increase in anxious behavior in the plus-maze test, both in rats and mice[27].

It is widely known that stress provokes an activation of the noradrenergic system, which gives rise to an increase in the release of noradrenaline and changes in the adrenergic receptors[28]. In situations of psychosocial stress, an increase has been observed in the tyrosine-hydroxylase enzyme in the locus coeruleus of subordinate rats[29], along with a reduction of alpha-2 adrenoceptors in the prefrontal cortex of animals subjected to defeat[30]. The down regulation of these autoreceptors occurs during the early phases of stress and reflects high levels of norepinephrine, while chronic stress produces an up regulation indicative of low levels of norepinephrine[31]. Postmortem studies of the brains of depressive human patients have revealed low levels of norepinephrine as well as an up regulation of alpha-2 adrenoceptors in a number of different cerebral area[32]. All these data suggest similar mechanisms are activated in both stressed animals and depressive humans.

Despite the fact that, as shown above, individuals subjected to social stress through defeat are more vulnerable to the development of depressive

anxious disorders, data exist in recent literature that suggest that maintenance of dominant status by more aggressive subjects may also lead to a stress response and exact a cost from such individuals. Numerous data show that the profile of dominant subjects is characterized by sympathetic hyperactivity, coupled with greater behavioral activation and more coping attempts than their subordinate counterparts[33]. Although dominant subjects also show an increase in corticosterone levels, adrenal and spleen weight, these changes are less pronounced than those observed in subordinate subjects[34].

In the present study, the stable losers, which were exposed to repeated experience of defeat, had showed an elevated level of anxiety. Treatment of these animals with diazepam produced an anxiolytic action and could enhance their competitive behavior; this had led to disruption of the dominance hierarchy.

Although the majority of data indicates that defeat increases anxiety indexes, in the case of experiences of victory in dominant individuals the results are not so consistent. It has been found that aggressive dominant animals show a more anxious behavior than their subordinate counterparts, presenting a coping strategy involving a greater degree of risk assessment behavior and avoidance of open arms in the plus-maze[35]. Thus dominant rats in situations of unstable social hierarchy showed a decrease in serotonin transporter bonding[36] that has been related to an increase in anxiety[37]. In contrast to this anxious effect of social stress in dominant rats, an anxiolytic effect of repeated victory has been observed in the absence of physical injury in rats exposed to aggressive encounters with an intruder[27]. Dominant rats in the resident-intruder model also show an anxiolytic profile in the open field, with greater locomotor and exploratory activity[38].

The present result indicated that no significant difference in competition scores was observed between unstable winners and losers; both had comparable competition scores and levels of anxiety. In contrast to the stable winners, the unstable "non-secure" winners suffered from a higher level of anxiety. Treatment with diazepam had an anxiolytic effect on these animals and produced a stable dominance hierarchy in 3 dyads; improving the status of anxiety was not enough to achieve an overall enhancement of the rather complex behavior of competition. Further studies are recommended to investigate the underlying mechanisms of these effects of diazepam on competitive behavior of animals.

The study of dominance using the dominant-submissive relationship seems to be a useful tool for understanding the mechanisms involved in this psychopathology. Drugs that have been found to be useful in mania, such as lithium, sodium valproate and carbamazepine are also effective inhibitors of dominant behavior in rats in the competition test[39].

It is concluded that social housing of rats could produce a dominance hierarchy; this model could induce a state of anxiety in these animals; treatment with an anxiolytic agent could improve their state of anxiety and enhance their competitive behavior.

REFERENCES

1- Valencia-Alfonso C, Feria-Velasco A and Luquin S et al.: **Revista De Neurolgia**; 38: 869-878 (2004).
2- Kessler R: **Annual Review of Psychology**; 48 191-214 (1997).
3- Brady K and Sonne S: **Alcohol Research and Health**; 23: 263-271 (1999).
4- Blanchard D, Sakai R and McEwen B et al.: **Behavioral Brain Research**; 58: 113-121 (1993).
5- Koolhaas J, DeBoer S and DeRuiter A et al. **Acta Physiologica Scandinavica**; 161: 69-72 (1997).
6- Blanchard D, Spencer R and Weiss S et al.: **Psychoneuroendocrinology**; 20: 117-134 (1995).
7- Buwalda B, Kole M and Veenema A et al.: **Neuroscience and Behavioral Reviews**; 29: 83-97 (2005).
8- Van Kampen M, Kramer M and Hiemke C et al.: **Stress**; 5: 37-46 (2002).
9- Miczek K: **Psychopharmacology**; 60: 253-259 (1979).
10- File S: **Behavior Brain Research**; 21: 195-202 (1986).
11- Mitchell J, Lewis R and Wilson M: **Research Communication Substance Abuse**; 9: 1-11 (1988).
12- Gentsch C, Lichtsteiner M and Feer H: **Behavior Brain Research**; 27: 37-44 (1988).
13- Malatynska E and Knapp R: **Neuroscience and Behavioral Reviews**; 29: 715-737 (2005).
14- Vogel J, Beer B and Clody D: **Psychopharmacologia**; 21: 1-7 (1971).
15- Shekhar A, McCann U and Meaney M et al.: **Psychopharmacology**; 157: 327-339 (2001).
16- Wittchen H, Kessler R and Beesdo K et al.: Generalized anxiety and depression in primary care: Prevalence, recognition, and management. **Journal of Clinical Psychiatry**; 8: 24-34 (2002).
17- Fuchs E, Kramer M and Hermes B et al.: **Pharmacology, Biochemistry and Behavior**; 54: 219-228 (1996).
18- Beitia G, Garmendia L and Azpiroz A et al.: **Brain, Behavior, and Immunity**; 19: 530-539 (2005).
19- Bartolomucci A, Pederzani T and Sacerdote P et al.: **Psychoneuroendocrinology**; 29: 899-910 (2004).
20- Sapolsky R: **Recent Progress in Hormone Research**; 48: 437-468 (1993).
21- Berton O, Aguerre S and Sarreieau A et al.: **Neuroscience**; 82: 147-159 (1998).
22- Meerlo P, Pragt B and Daan S: **Neuroscience letters**; 225: 41-44 (1997).
23- Avgustinovich D, Alekseyenko O and Koryakina L: **Life Sciences**; 72: 1437-1444 (2003).
24- Gorman J: **Depression and Anxiety**; 4: 160-168 (1997).
25- Avgustinovich D, Gorbach O and Kudryavtseva N: **Physiology and Behavior**; 61: 37-43 (1997).
26- Corbett R, Hartman H and Kerman L et al.: **Pharmacology, Biochemistry and Behavior**; 45: 9-17 (1993).

ISSN 1110-5089

27- Haller J. and Halasz J: Aggressive Behavior; 26: 257-261 (2000).
28- Stanford S: Central noradrenergic neurons and stress. Pharmacology and Therapeutics; 68: 297-342 (1995).
29- Watanabe Y, Mckittrick C and Blanchard D et al.: Molecular Brain Research; 32: 176-180 (1995).
30- Meyer H, Palchaudhuri M and Scheinin M. et al.: Brain Research; 880: 147-158 (2000).
31- Flugge G: A Survey of Cell Biology; 195: 145-213 (2000).
32- Garcia-Sevilla J, Escriba P and Ozaita A et al.: Journal of Neurochemistry; 72: 282-291 (1999).
33- Creel S: Trends in Ecology and Evolution; 16: 491-497 (2001).
34- Blanchard R, Yudko E and Rodgers R et al.: Behavioral Brain Research; 58: 155-165 (1993).
35- Kudryavtseva N, Bondar N and Avgustinovich D: Behavioral Brain Research; 133: 83-93 (2002).
36- McKittrick C, Magarinos A and Blanchard D et al.: Synapse; 36: 85-94 (2000).
37- Caspi A, Sudgen L and Taylor A et al.: Science; 301: 386-389 (2003).
38- Bartolomucci A, Palanza P and Gaspani L et al.: Physiology and Behavior; 73: 401-410 (2001).
39- Malatynska E, Goldenberg R and Shuck L et al.: Pharmacology; 64: 8-17 (2002).

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تأثير العلاج بمادة الديازيبام علي السلوك التنافسى فى ذكور الجرذان وقياس مستوى القلق فى الجرذان المتنافسة

السيد العوضى

قسم الأدوية والسموم- كلية الصيدلة- جامعة قناة السويس- الإسماعيلية- مصر

تهدف الدراسة الحالية لدراسة تأثير مادة الديازيبام على السلوك التنافسي في ذكور الجرذان وقياس مستوى القلق فى الجرذان المتنافسة.

أجريت الدراسة على أربعة وأربعين من جرذان التجارب المحرومة من الطعام ، كونت هذه الحيوانات 22 زوجا تم تقسيمهم إلى مجموعتين حسب نتائج المنافسة على الطعام ، مجموعة ثابتة الترتيب (12زوجا) ، ومجموعة متغيرة الترتيب (10أزواج)، تم حقن هذه الحيوانات بمادة الديازيبام (0.5 مجم /كجم) فى التجويف البريتونى قبل الاختبار بنصف ساعة ثم قياس مستوى القلق فى نصف كل مجموعة وأجريت المنافسة على الطعام على النصف الآخر.

أظهرت النتائج أن معالجة كل من الفائزين والخاسرين بمادة الديازيبام قد أدى إلى تغيير نتائج المنافسة فى هذه الحيوانات فقد استطاع الديازيبام أن يرفع من مستوى السلوك التنافسى للحيوانات مما أدى إلى ثبات الترتيب فى المجموعة الغير ثابتة ومن ناحية أخرى أظهرت النتائج ان الفائزين والخاسرين فى المنافسة على الطعام يعانون من مستويات مختلفة من القلق وقد خفض الديازيبام من مستوى القلق فى الجرذان الخاسرة فى التنافس.

تخلص الدراسة إلى أن تسكين الجرذان فى مجموعات يؤدى إلى ترتيب هذه الحيوانات فى مجموعاتها تنافسيا إلى فائزين وخاسرين وان هذا النموذج يخلق نوعا من القلق فى هذه الحيوانات وان العلاج بمادة الديازيبام يؤدى إلى تحسين حالة القلق ورفع مستوى السلوك التنافسى فى هذه الحيوانات.