Antidepressant-Like Effects of Modulating Light:Dark Cycle on Female Wistar Rats in the Forced Swim Test

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ABSTRACT

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T.Alper Karslı

Two experiments aimed to determine the antidepressant-like efficiency of modulating Light:Dark cycle in either Light:Light or Light:Dark direction for one session. In the first experiment, temporal extent of the antidepressant-like protective effects of light exposure during the dark phase of the Light:Dark cycle for one session on the behavioural performance in female Wistar rats was determined. Fifty-six female Wistar rats were randomly allocated either to one of 3 experimental or 4 control groups, which underwent two Forced Swimming Test (FST's) sessions, separated by 24 hours, after either 24, 96 or 168 hours following the offset of light exposure. Animals in the fourth control group, called as the no treatment group, were kept in vivarium and underwent only the FST trials without any manipulation. Antidepressant-like protective effects of light were observed to extend to 168 hours. In the second experiment, antidepressant-like efficiency of converting Light:Dark cycle into Dark:Dark cycle in FST was measured. Sixteen female Wistar rats were randomly allocated to the either experimental or control group. Subjects underwent FST 1 24 hours later following the offset of the Dark:Dark condition followed by FST 2 24 hours later. Experimental group was less immobile in FST when compared to the control group, which indicated an antidepressant-like efficiency for Dark:Dark treatment.

ÖZET

Aydınlık:Karanlık Döngüsünün Modülasyonunun Dişi Wistar sıçanlarda Yüzme Testindeki Antidepresan Etkileri

T.Alper Karslı

Bu çalışma kapsamındaki iki deneyde 24 saatlik aydınlık-karanlık döngüsünü aydınlık-aydınlık veya karanlık-karanlık yönünde değiştirmenin antidepresan etkinliği incelenmiştir. Birinci deneyde aydınlık-karanlık döngüsünün karanlık fazında bir gecelik verilen 12 saatlik ışık pulsünün dişi Wistar sıçanların 24 saat aralıkla uygulanan iki yüzme testindeki (FST) performansı üzerine etkinliği ve bu etkinliğin zamansal sınırları araştırılmıştır. Bu deneyde 3 adet deney grubu, bu deney gruplarının herbirinin kendi kontrol grubu ve bir adet de herhangi bir manipulasyona maruz kalmamış olan temel kontrol grubu olmak üzere 7 gruba random olarak dağıtılmış olan 56 adet dişi Wistar sıçan kullanılmıştır. Deneysel manipulasyonun sona ermesinden, bağlı bulundukları gruba göre, 24, 96 ya da 168 saat sonra yüzme testine tabi tutulmuşlardır. İstatistik analizler ışığın koruyucu antidepresan etkinliğinin ışık pulsünün sona ermesinden 168 saat sonra bile etkili olduğunu göstermiştir. İkinci deneyde aydınlık:karanlık döngüsünü karanlık:karanlık döngüsüne çevirmenin antidepresan etkinliği araştırılmıştır. Bir tane deney ve bir tane de kontrol grubuna random esasına göre dağıtılmış olarak 16 adet dişi Wistar sıçan kullanılmıştır. İstatistik analizler deney grubunun kontrol grubuna oranla ikinci yüzme testinde yüzme testinde anlamlı düzeyde daha az hareketsiz olduğunu göstermiştir.

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INTRODUCTION

Circadian Rhythms

Exhibiting approximately 24-hour rhythms (circadian rhythms) is a common biological feature of eukaryotic organisms, which gives them the chance of adapting to changing environmental conditions (Wehr and Wirz-Justice, 1982). This rhythmicity in endocrinal, physiological and behavioral functions of organisms is a unique property of eukaryotes that is observed at the levels of organism, tissue and cell (Ding et al, 1997). Pacemakers such as the suprachiasmatic nuclei (SCN) generate circadian rhythms (Sumova et al, 1998). Under stable conditions, circadian rhythms do not deviate marginally from 24 hours, since they are adjusted to 24 hours with regard to the external time-cues called "zeitgebers". Zeitgebers help organisms entrain to the day-night cycle and this entrainment process is mediated by neural pathways such as the retinohypothalamic tract that connects the retina to the SCN (Best et al, 1999).

In organisms, observed rhythms are often assumed to be generated by "oscillators" or clock-like mechanisms that are able to measure time in the absence of external cues (Bunney and Bunney, 2000). The principal circadian oscillators of mammals are the hypothalamic suprachiasmatic nuclei (SCN) and the dominant signal that coordinates internal time with environmental changes is light (Reghunandanan, Reghunandanan and Singh, 1993). While it is true that an endogenous biological rhythm is generated by an oscillator within the organism, under normal conditions the oscillator is usually synchronised or "entrained" by either periodic environmental cues or by other endogenous biological rhythms and synchronised or "coupled" by varying degrees to other oscillators (Pittendrigh, 1988). In the absence of an entrained oscillator, its rhythm is considered as "free running" at the oscillator's natural frequency, or at a frequency compatible with its

coupling to other oscillators (Bunney and Bunney, 2000). Circadian oscillators controlling rhythms are commonly reset or entrained daily by 24 hours cues resulting in rhythms with a 24 hours periodicity (Ding et al, 1994).

Circadian System Disturbances in Mood Disorders

Humans with mood disorders such as depression often exhibit disturbances in their circadian rhythms (Solberg, Horton and Turek, 1999). Animal studies as well as clinical studies indicate that a disturbance in the circadian rhythm (lengthened free running period for instance) is observed along with other behavioral impairments considered as the manifestation of the state called behavioral depression (Stewart et. al., 1990a). In the literature, under 24 hours normal environmental L:D cycle, basic types of circadian abnormalities have been associated with major kinds of affective disorders (Solber, Horton and Turek, 1999).

One of the most prominent circadian abnormalities observed in patients suffering mood disorders is the internal desynchronisation of the rhythms, in which at least one biological rhythm that is completely or partially uncoupling from other rhythms, continually changes its relationships with the external time-cues (Madden et al, 1996). A complete internal desynchronisation of the circadian rhythms generally coincides with especially bipolar depressive symptoms; however, relatively constant but abnormal phase relationships between different rhythms and environment are also observed in bipolar depressives (Rosenthal et al, 1988).

Major Affective Disorders Associated with Circadian Abnormalities: Depression and Seasonal Affective Disorder

Unipolar Depression

Numerous studies conducted over a period of more than 25 years provide data to suggest that a subgroup of nonseasonally depressed patients may also have a circadian rhythm disorder (Hallonquist, Goldberg and Brandes, 1985). Meta-analysis of data

indicates that circadian rhythm disorders, which are generally manifested during sleep such as changes in body temperature and cortisol secretion, are among the most considerable parameters for distinguishing depressives from nondepressed individuals (Brotto et al, 1998; Wehr and Wirz-Justice, 1982). For example, phase advance in nocturnal cortisol secretion relative to sleep onset in depressive patients provides clinical evidence that is compatible with a disturbance in circadian rhythms (Solberg, Horton and Turek 1999). It is also suggested that depression involves a weaker coupling process between internal pacemakers and has abnormal sensitivity to environmental cues such as light, which is the major zeitgeber for mammals (Bunney and Bunney, 2000).

Normal individuals have maximum temperatures in the afternoon and minimum temperatures between 4:00 and 6:00 a.m., which is the second half of the sleep period; however in depressed patients both temperature maxima and minima are so advanced that temperature decreases during wakefulness and increases during sleep (Madden et al, 1996). According to the internal coincidence model of unipolar depression, sleeping during the ascending portion (deemed as the critical phase) of the circadian temperature rhythm is a characteristic circadian rhythm disorder in depressive patients and may actually induce depression (Madden et al, 1996).

Bipolar Depression

In the literature, a few rapidly cycling bipolar depressives have been reported to show a relatively complete desynchronisation in coupling of circadian rhythms (Wehr and Wirz-Justice, 1982). Under normal environmental conditions, circadian rhythms of body temperature, pulse rate and urinary constituents, but not sleep-wakefulness cycle, are shown to free run with periods shorter than 24 hours in such individuals (Solberg, Horton and Turek, 1999). Observations indicate that this type of a circadian abnormality in bipolar depressives results in a peak depression level when the temperature peak occurs between midnight and 6:00 a.m., however results in manic state between 6:00 p.m. and midnight

(Rosenthal et al, 1988). It is also reported that spontaneous switches from depression to mania in bipolar depressive patients coincide with one or more double-length (48 hours) sleep-wake cycles (Brotto et al, 1998; van Esseveldt, Lehman and Boer, 2000). These 48 hours sleep-wake cycles typically reflect one night's total insomnia and can result from the strong and weak oscillators briefly uncoupling because of abnormally different intrinsic periods, which in turn leads bipolar patient to manic state (Brotto et al, 1998).

In addition, some other observations indicate considerable phase advances in sleep rhythms, REM propensity for instance, which is observed as reduced latency and increased length of first REM period during the switch from depression to mania (Wehr and Wirz-Justice, 1982). Early awakening from sleep concurrent with apparent redistribution of REM sleep to earlier portions of sleep period is in accordance with findings indicating abnormal phase advances in these patients and can be mimicked in nondepressive individuals by phase advancing manipulations (Pittendrigh, 1988). A relative advance of the strong oscillator in comparison to weak oscillator is reported in both bipolar and unipolar depressive patients (Madden et al, 1996). Normal individuals that maintain internal synchronisation of free running rhythms in the absence of 24 hours cues are also shown to exhibit phase advances of REM sleep and cortisol secretion in addition to temperature (Van Esseveldt, Lehman and Boer, 2000).

Seasonal Affective Disorder

Seasonal affective disorder (SAD) is a mood disorder characterised by recurrent depressions that relapse the same time, especially in winter months, every year (Hallonquist, Goldberg and Brandes, 1985). These depressive phases are associated with symptoms such as hypersomnia and carbohydrate craving, which are compared to aspects of "hibernation" (Madden et al, 1996). The circadian abnormalities in SAD include sleep disturbances, increases in the minimal core body temperature and disturbances in cortisol level (Wehr and Wirz-Justice, 1982). Phase delay in core body temperature, cortisol and

melatonin secretion in some patients suffering form SAD is also reported (Hallonquist, Goldberg and Brandes, 1985). It is hypothesized that in many SAD patients environmental and social zeitgebers such as sleep and meal times act as a time-cue and provide the patients with opportunity of adapting to daily activities successfully, which in turn helps to mask the expression of their abnormal circadian pacemaker activity (Pittendrigh, 1988).

Effects of Light Exposure on the Circadian System as an Antidepressant Agent

The concept of light exposure, especially in SAD patients, by using artificial light sources for therapeutic purposes can be traced back to early decades of the 20th century (Rosenthal et al, 1988). However, although past studies generally involved SAD patients as subjects and therapeutic efficiency of light was thought to be limited to SAD cases, contemporary studies indicate that nonseasonal depression may also result from inadequate light exposure at critical times of the day (Kripke et al, 1978).

In human studies there is a controversy as to which time of day is the most effective for light exposure in alleviating depressive symptoms. In many studies, especially in SAD patients, light exposure, when applied in the morning, is found to be superior to light exposure applied in the evening in terms of therapeutic efficiency (Lewy et al, 1987; Rosenthal, 1988). The main theoretical basis accounting for the superiority of light exposure applied in the morning is the "phase shift hypothesis". According to this hypothesis, antidepressant-like efficiency of artificial bright light depends on shifting the phase of circadian rhythms in SAD patients; light exposure applied in the morning advances the circadian rhythms (phase advances) and light exposure applied in the evening delays the circadian rhythms (phase delays) (Lewy et al, 1987). Since most of the SAD patients are known to be abnormally phase delayed, they respond to light exposure applied in the morning, which phase advances their circadian rhythms. On the other hand, unlike SAD patients of Lewy (1987), a majority of nonseasonally depressed patients and bipolar

depressives display phase advances in their circadian rhythms, which are phase delayed by antidepressant treatments.

SAD patients seem to benefit from very short light exposures (even as short as 15-minutes) in the morning. For instance, in a study on SAD patients by Partonen (1994) antidepressant effect of morning light exposure to two groups of subjects for 15 minutes or 1 hour for two weeks was compared. Results indicated that after the period of two weeks of light treatment, the depressive symptoms of subjects in the experimental groups were alleviated significantly in comparison to control subjects. Also, the effectiveness of light treatment was positively correlated with the duration of exposure; the group that underwent 1 hour light exposure for two weeks was significantly less depressive than the group underwent light exposure for 15 minutes. However, this difference gradually disappeared and at the end of two weeks there was no significant difference between these two experiment groups on the basis of their depression level. In another study, conducted by Lewy et al (1987), exposing SAD patients to light in intensity of 2500 lux also led to antidepressant-like results, which were correlated with melatonin suppression when applied in the dark phase of the L: D cycle.

There is a considerable methodological difference between these two studies on the basis of treatment conditions. The difference is that in the study by Lewy et al (1987), there were two additional experimental groups. One of these groups was exposed to light only in the evening and the other group was exposed to light in both morning and evening. Intensity and duration of the light exposure were the same for all experimental groups. In this study, antidepressant effect of light exposure applied in the morning was found to be superior to light exposure applied in the evening. Furthermore, light exposure applied both in morning and evening was more effective than applying only in the evening but less effective than applying only in the morning.

In a study by Wirz-Justice et al (1993) on SAD and bipolar depressive patients, no significant effect of the time of light exposure was found. Subjects were exposed to light of 2500 lux in intensity at either 7:00 a.m. or 10:00 p.m. for one week and results indicated that effectiveness of the light treatment does not differ with regard to the time of exposure. Meta-analysis indicated that applying light exposure to low-depressed SAD patients (patients scored lower than 13 points on the Hamilton Depression Scale) in the morning is more beneficial than applying in the evening, however for high-depressed SAD patients (patients scored higher than 13 points on the Hamilton Depression Scale) such a difference between applying light exposure in the morning or evening is not observed (Wirz-Justice et al, 1993).

Assessing the Antidepressant-Like Effects of Light Exposure in Animal Studies: The Forced Swimming Test and Behavioral Despair Paradigm

In human studies, antidepressant-like efficiency of light treatment is generally measured and assessed by using depression scales like Hamilton Depression Scale (HAM-D), which depends on self-reports (Rosenthal, 1988; Wirz-Justice et al, 1993). Such self-report techniques are vulnerable to various extraneous variables including social interactions and possible placebo effect of light treatment (Pittendrigh, 1988; Wirz-Justice et al, 1993). However, animal studies provide the opportunity to measure the antidepressant-like efficiency of light treatment under more controlled conditions, which exclude many of the confounding variables in human studies that can contaminate the results (Willner, 1990). Animal studies, in addition to chance of observing the consequences of antidepressant treatments such as light at behavioral level, provides us with the opportunity to investigate and make inferences about the neurophysiological and neurochemical mechanisms underlying antidepressant efficiencies of those treatments (Porsolt et al, 1978; Willner, 1990).

The forced swim test (FST) is a widely used depression model in animal studies since 1977 (Abel, 1993; Porsolt et al, 1977; Willner, 1990) for screening antidepressant efficiencies of a variety of antidepressant agents such as tricyclic antidepressants, which exert their antidepressant efficiency by acting on both NA and 5-HT systems (Connor, Kelliher and Leonard, 1997; Porsolt et al, 1977; Willner, 1990). The forced swim test provides us with a basic animal model of depression with its behavioral and physiological components (Willner, 1990). Furthermore, the FST can be used for both inducing behavioral depression and screening the efficiencies of antidepressant agents (Connor et al, 2000).

The FST is a two-step test (Porsolt et al, 1977). In the first trial (FST 1), the subject is forced to swim for 15 minutes in a standard plexiglass cylinder, which induces a prolonged immobile posture called as behavioral despair in the second 5 minutes test (FST 2) conducted 24 hours later (Willner, 1990). It is accepted in the literature that behavioral despair results from loosing the hope of escaping from the aversive test situation (Porsolt et al, 1978). In the second trial (FST 2) carried out 24 hours later after FST 1, subject swims in the same plexiglass cylinder for 5 minutes and the difference in its duration of immobility across FST 1 and FST 2 is considered as the main parameter (Abel, 1993). Non-protected subjects (via antidepressant drug treatment and/or light exposure) display a longer duration of immobility in FST 2 in comparison to duration of immobility they displayed in FST 1 and this increase in duration of immobility is considered as the sine qua non of behavioral despair (Porsolt et al 1977; Willner, 1990). In FST 2, a decrease in escape-oriented behaviors (diving and jumping behaviors for instance) and headshaking behavior generally coincides with prolonged immobility in the subjects that are not protected from behavioral despair by antidepressant treatment (Willner, 1990).

Neurochemical changes that occur in certain brain regions of the animals experiencing behavioral despair by virtue of being exposed to the FST is similar to the neurochemical

alterations in the CNS of depressive patients (Connor et al, 2000). FST is known to decrease cortical and increase amygdaloidal 5-HT turnover (Reneric, Bouvard and Stinus, 2001). Hypothalamo-adrenal axis (HPA) is also activated by the FST and can be attenuated by administration of tricyclic antidepressants (TCA's) and monoaminoxidase inhibitors (MAOI's) (Duncan et. al, 1996;Reneric, Bouvard and Stinus, 2001). Along with HPA axis activation, increased serum corticosterone, decreased serum glucose and adrenal ascorbic acid are among well-established neuroendocrinal/biochemical consequences of the FST induced stress, which can be reverted to the normal state by light-treatment as well as antidepressant drug treatment (Connor, Kelly and Leonard, 1997; Connor et al, 2000). It is also known that in the FST, serum corticosterone level increases at each duration of testing in the manner that increaments are generally higher than the previous duration and there is a negative correlation between the water temparature and increase in serum corticosterone levels (Abel, 1993).

5-HT and NA systems have different behavioral effects in the FST, for instance increased 5-HT turnover results in increased amount of escape-oriented behaviors (diving and jumping for instance), however increased NA turnover results in decreased duration of immobility (Detke, Rickles and Lucki, 1995). Inhibition of the re-uptake of 5-HT and NA is shown to decrease immobility and increase escape-oriented behaviors and headshaking behavior; for instance, desipramine, a tricyclic antidepressant, is known to reduce immobility in the FST by inhibiting NA re-uptake into the presynaptic neuron (Espejo and Minano, 1999; Reneric, Bouvard and Stinus, 2001).

As a general evaluation, exposing animals to swim stress in the FST induces alteration in behavior, which is considered to model prominent behavioral, circadian and physiological symptoms of depressive state (Porsolt et al, 1978; Willner, 1990). For these reasons, the FST is a convenient experimental tool for modeling depression with its all components in a practical way.

Antidepressant-Like Efficiency of Light Exposure in the FST

Animal data on the antidepressant-like effects of light exposure observed in the FST is in the same direction with data derived from human studies; light exposure reduces the increase in immobility duration of experimental subjects in FST 2 in comparison to their duration of immobility in FST 1. For instance, in a study by Hernandez et al (1999) male Wistar rats were exposed to a long photoperiod (12 hours of light exposure during the subjective night) for two weeks and this elongated photoperiod treatment was able to reduce the increase in duration of immobility in the FST 2. In this study antidepressant effects of light treatment was compared with two antidepressant drugs (imipramine and desipramine) and was found to be as effective as these two tricyclic antidepressants. However, as expressed by the authors of this study, the FST used in this study was not in accordance with the standards, since it was not conducted in a cylinder but in a plexiglass cube. Results of another study (Arushanian, 1999) also indicated that antidepressant-like protective efficiency of 14 days of light treatment in male rats is as effective as an antidepressant drug (imipramine).

The studies above used light exposure for durations as long as 2 weeks, which is similar to that of human studies. However, it was shown in our laboratory that 12 hours of light exposure during the dark phase of the L:D cycle for one session (converting 12L:12D cycle into 12L:12L for 24 hours) has antidepressant-like protective effects on female Wistar rats underwent subsequently applied FST. In a study by Yılmaz et al (2000) rats, which were exposed to a single 12 hours light pulse during their subjective night, were less immobile and performed significantly more diving, jumping and headshaking behaviors in the FST. In this study, antidepressant-like protective effect of 12 hours of light exposure for one night were shown to be observed 36 hours later following the offset of the treatment. The finding of this study that a single 12 h light pulse exposed during the dark phase of the L:D cycle can exert antidepressant-like efficiency in the FST even after 36

hours later following the offset of the exposure is the first in the literature. However, temporal limits of the ameliorative effects of 12 h light pulse during the dark phase of the L:D cycle has not yet been studied. It is possible that antidepressant-like protective efficiency of 12 hours of light exposure during the dark phase of the L:D cycle for one night can be observed beyond 36 hours.

Effects of Dark Exposure on the FST

Nocturnal animals seem to be less stressed in behavioral tests such as FST when conducted under nocturnal conditions (Kelliher et al, 2000). For example in a study by Kelliher et al (2000) rats were less stressed in terms of reduced agitation against the experimenter and spent a shorter period of time in escape-oriented activity when compared to FST's carried out under diurnal conditions.

Keeping animals under continuous darkness is shown to have protective effects in such behavioral tests, which probably relies on continuously secreted melatonin from the pineal gland (Shaji and Kulkarni, 1998). Continuous darkness, like L:L condition, leads to circadian and neurochemical alterations in organisms (Goodwin et al, 1999; Stewart et al, 1990a) However, it is not known whether exposing subjects to a prolonged dark cycle (12D:12D) exerts antidepressant efficiency in the FST carried out under diurnal conditions.

Aim of the present study

The first experiment was conducted to determine whether antidepressant-like protective effects of 12 h light pulse for one session on behavioral despair paradigm in the FST can be observed beyond 36 hours limit observed in the previous study (Yılmaz et al, 2000) in our laboratory. A second experiment was conducted to observe the effects of the opposite (D:D condition for 24 hours) of treatment used in the first experiment on behavioral despair paradigm in the FST.

EXPERIMENT 1

Materials and Method

Subjects: A total of 56 female Wistar rats from the breeding colony in the Psychobiology Laboratory weighing 185-210 grams was used in this study. Subjects were kept in plastic cages in separate groups housed eight to a cage. Subjects were maintained on 12L:12D (lights on at 7:00 am) schedule in the vivarium with free access to food and water.

Groups and Experimental Conditions: A total of 7 groups were run. There were 3 experimental groups (one-day delay, 4-day delay and 7-day delay experimental groups) and 3 control groups (one-day delay, 4-day delay and 7-day delay control groups). A no treatment group took place as a fourth control group. This group also consisted of 8 animals chosen randomly from separate cages like other groups but subjects from this group were allowed to live their routine L:D cycle in the vivarium and were not put in the wooden chamber. Subjects in the experimental groups were exposed to light for 12 hours during the dark phase of their L:D cycle in an insulated and ventilated wooden chamber, duration of time for light exposure was the same for all experimental groups. However, the interval of time between the offset of the treatment and the testing session was different for each of the groups. The first experimental group (one-day delay experimental group) was tested in the FST 24 hours after the offset of light exposure. For the 4-day delay experimental group the time passed between the offset of the treatment and testing session in the FST was 96 hours and for the 7-day delay experimental group this period was 168 hours. Subjects in the control groups of the experimental groups were also put in the insulated and ventilated wooden chamber as for the experimental groups and stayed there for the same periods of time as their respective experimental group. However, they were not exposed to light during the dark phase of their L:D cycle (19:00-07:00) and were

allowed to live their routine L:D cycle in the wooden chamber as they had done in the vivarium. One-day delay control group was tested in the FST 24 hours later after being taken out of the wooden chamber. For the 4-day delay control group time passed between being taken out of the wooden chamber and test session was 96 hours and for the 7-day delay control group this period was 168 hours.

Experimental Procedure:

Light Exposure: For the pre-treatment (for either experimental or control conditions) all subjects were individually placed in an sound-light isolated and ventilated wooden chamber (90x70x50 cm) 10 min. prior to the onset of the dark phase (18:50) of the L:D cycle in small plastic cages with food and water available *ad libitum*. The experimental groups received light by means of a 25 watt incandescent bulb attached to the ceiling of the wooden chamber during the dark phase of the 12L:12D cycle for 12-hours (19:00-07:00) and light in the wooden chamber continued to be on until they were taken out of the chamber at 15:00 h. Subjects from the control groups were also individually put in the wooden chamber 10 minutes prior to the onset of the dark period of the L:D cycle like the experimental groups but they were not exposed to light between 19:00-07:00 h. For the control groups, in accordance with that of the vivarium, light was on in the wooden chamber at 07:00 h. All experimental and control groups (except no treatment group) were kept in the wooden chamber for the same amount of time (32 hours). All subjects were taken out of wooden chamber at 15:00 h and then were put back into their homecages.

FST Procedure: FST's were conducted in a standard clear plexiglas cylinder 45cm in height and 30cm in width filled with water to a depth of 15cm at 25 c. All subjects were placed individually to into small plastic cages and were introduced to the test room 10 minutes prior to the initiation of the test session. Each of the subjects underwent two distinct swimming tests (FST 1 and FST 2) individually. FST 1 (15 minutes) was followed by FST 2 (5 minutes) 24 hours later under the same experimental conditions. In each FST

trials, duration of immobility, diving, jumping behaviors and headshake scores of each subject were assessed for statistical analysis. Immobility was defined as floating. Diving was defined as immersion of the whole body into water and jumping was defined as attempt to escape from the cylinder with at least the upper half of the body out of the water. Headshake behavior of the subjects was also scored for statistical analysis. After the end of each FST session, each subject was allowed to dry for 30 minutes under a lamp in a cage covered with tissue paper. All tests were recorded via a videocamera.

Results and Discussion of Experiment 1

One-way ANOVA was used for duration of immobility and followed by LSD for post-hoc comparisons when a significant difference among the groups was attained. Other behavioral measurements (diving, jumping and headshaking) were analysed using Wilcoxon Matched-Pairs Signed-Ranks Test and Kruskall-Wallis one-way ANOVA tests.

Each group's diving, jumping and headshaking behavior scores observed in the initial 5 minutes of FST 1 were compared with its own diving, jumping and headshaking behavior scores observed in FST 2 separately by using Wilcoxon Matched-Pairs Signed-Ranks Test. Duration of immobility of the subjects in the initial 5 minutes of FST 1 was compared with their own duration of immobility in FST 2 by using one-sample t-test. For detecting possible significant differences among the diving, jumping and headshaking behavior scores of the groups, Kruskall-Wallis one-way ANOVA was conducted for both FST 1 and FST 2. The results are shown in Fig. 1-4 and Tables 1-5 (Appendix1a).

Results of FST 1

Duration of immobility of the groups was not significantly different in FST 1 ($F(6,49)=1.769$, $P>0.05$) (Table1-a). Non-parametric Kruskall-Wallis One-Way ANOVA did not reveal any significant difference among the groups for diving, jumping or headshaking behavior in FST 1 (chi-square= 4,82 df= 6 sig.= 0.612, $p>0.05$ for diving, chi-

square=11,295 df=6 sig.= 0.800, p>0.05 for jumping and chi-square=11.240 df=6 sig.= 0.810, p>0.05 for headshaking) (Table1-b).

Results of FST 2

One-way ANOVA indicated a significant treatment effect among the groups in duration of immobility (F(6,49)=3.421, P<0.05) (table-2a). Post-hoc analysis (LSD) (table-Tb) indicated that, One-day delay experimental group was significantly less immobile than one-day delay control (0.038), 4-day delay control (0.045) and the no treatment groups (0.001). The 4-day delay experimental group was significantly less immobile than only the no treatment group (0.030). The 7-day delay experimental group was significantly less immobile than one-day delay control (0.046) and no treatment groups (0.001). In addition, 7-day delay experimental group was less immobile than 4-day delay control group in FST 2 at a very close level to be statistically significant (0.056). There was no significant difference among the immobility duration of the experimental groups. The only significant difference among duration of immobility of the control groups in FST 2 was between 7-day delay control group and the no treatment group (0.004) (Table-2b).

Non-parametric Kruskall-Wallis One-Way ANOVA did not indicate any significant difference among the groups for diving, jumping and headshaking behavior scores in FST 2 (Table-3).

Comparisons between FST 1 and FST 2

One-sample t-test analysis was carried out for comparing the duration of immobility of the groups in FST 2 with their own duration of immobility in FST 1. One-day delay experimental (t=1.296, df=7, p>0,05) and 7-day delay experimental (t=1.692, df=7, p>0.05) groups were not significantly more immobile in FST 2 than they were in FST 1 (Table-4). The rest of the groups displayed significantly longer duration of immobility in FST 2 than immobility duration they displayed in FST 1 (for the one-day delay control group t=4.212, df=7 p<0.05, for the 4-day delay experimental group t=2.997, df=7, p<0.05,

for the 4-day delay control group t=2.975, df=7, p<0.05, for the 7-day delay control group t=3.048, df=7, p<0.05 and for the no treatment group t=5.394, df=7, p<0.05) (Table-4).

Insert Figure-1 About Here

Diving, jumping and headshaking behavior scores of each group in FST 1 and FST 2 were compared with its own scores separately by using Wilcoxon Matched-Pairs Signed-Ranks Test (Tables-5a, b and c). Except for the 4-day delay experimental group, a significant difference was not found in diving and jumping behavior scores between FST 1 and FST 2 for any of the groups. The 4-day delay experimental group showed significantly (z=-2,3664 p=0,018, p<0,05) less diving behavior in FST 2 in comparison to its own diving score in FST 1 (Table-5a).

Insert Figure-2 and Figure-3 About Here

Wilcoxon Matched-Pairs Signed-Ranks Test indicated significantly less headshaking behavior for the 4-day delay control and 7-day delay experimental groups in FST 2 than they did in FST 1 (z=-2,5205 two-tailed p=0,0117, p<0,05 for the 4-day delay control group and z=-2,0284 two-tailed p=0,0425, p<0,05 for the 7-day delay experimental g roup (Table5-c).

Insert Figure-4 About Here

Discussion

Results indicated that a single 12 h light pulse during the dark phase of the L:D cycle has an antidepressant-like effect in the FST. This antidepressant-like protective effect was observed for the one-day delay and 7-day delay experimental groups; Subjects in these groups were not significantly more immobile in FST 2 in comparison to their own duration

of immobility in FST 1. The one-day delay experimental group was significantly less immobile than one-day delay control, 4-day delay control and the no treatment groups. The 7-day delay experimental group was also less immobile than the one-day delay control and no treatment groups. In addition, there was no significant decrease in diving and jumping behaviors of the one-day delay and 7-day delay experimental groups across FST 1 and FST 2. There was no significant difference among the groups in any of the parameters in FST 1 indicating that our results cannot be a consequence of possible motor problems of the subjects in the control groups and 4-day delay experimental group.

By virtue of 12 h light pulse during the dark phase of the 12L:12D cycle for one night, subjects in the experimental groups lived under 12L:12L condition for 24 hours. On a circadian basis, light exposure is known to exert antidepressant-like efficiency by phase shifting the circadian rhythms (Lewy et al, 1987). However, effects of the opposite of this treatment, being exposed to D:D condition for a period of time as short as 24 hours on FST carried out under diurnal conditions, has not been studied up to date.

EXPERIMENT 2

Twelve hours of light exposure during the dark phase of the L:D cycle for one night (converting L:D cycle to L:L for 24 hours) showed antidepressant-like efficiency in FST-induced behavioral despair in the first experiment. A second experiment was conducted to observe whether exposing subjects to 12 hours of darkness during the light phase of the L:D cycle for one day (converting 12L:12D cycle to 12D:12D condition for 24 hours) has antidepressant-like protective effects on the FST-induced behavioral despair paradigm like 12L:12L condition.

Materials and Methods

Subjects and Groups: A total of 16 female Wistar rats weighing 190-220 grams was used in this study. Animals were kept in plastic cages housed eight to a cage. Food and water were available *ad libitum*. Half of the subjects (experimental group) were kept in

continuous darkness in an isolated chamber for 24 hours (D:D condition). Subjects in the control group were put in the same chamber, but they were maintained on the usual L:D cycle (12h.light:12h.dark) during this period.

Experimental Procedure

D:D Treatment: Animals in the experiment group were put in the ventilated wooden chamber (90x70x50) insulated from extraneous light and sound 10 minutes prior to the onset of the dark period of the L:D cycle (18:50) and kept there for 24 hours and 10 minutes. This treatment converted the L:D cycle in vivarium to D:D for the experimental animals for one day. Twenty-four hours after the offset of this treatment, subjects in the experimental group underwent FST 1. Control subjects were kept in the insulated and ventilated wooden chamber for the same duration as for the experimental group, but they were maintained on the 12L:12D condition as they had done in the vivarium. Control animals also underwent FST 1 24 hours after they were taken out of the wooden chamber.

FST Procedure: FST procedure was the same as Experiment 1. Animals underwent FST for two consecutive days (FST 1 and FST 2) in a plexiglas cylinder 45cm in height filled with 25°c water to a depth of 15cm. Following each FST session, all animals were allowed to dry for 30 minutes under a lamp in a cage covered with tissue paper. All experiment were recorded via a videocamera.

Results and Discussion of Experiment 2

Independent Samples t-Test was conducted for comparing the immobility duration of the experimental and control group. One-Sample t-Test was conducted for comparing each group's immobility duration in initial 5 minutes of FST 1 with its own duration of immobility in FST 2. Diving, jumping and headshaking scores were separately analysed by using Mann-Whitney U non-parametric test. Results are shown in Fig.5-8 and Tables 1-6 (Appendix-2b).

Results of FST 1

The control and experimental groups were not significantly different in duration of immobility in FST 1, ($t=-3.4810$ $df=14$, $p>0.05$), however the experimental group was nearly significantly less immobile than the control group (0.056) (Table-1).

Mann-Whitney U-Wilcoxon Sum W Test did not indicate any significant differences between the experimental and control group for diving, jumping and headshaking behaviors (Table-2).

Results of FST 2

The experimental group was significantly less immobile than the control group in FST 2 ($t=-2.2650$ $df=14$, $p<0.05$) (table-3). Mann-Whitney U Wilcoxon Sum W Test did not reveal any significant difference for diving and jumping behaviors (Table-4). Experimental subjects emitted significantly more headshaking behavior than controls, ($z=-2.0524$, $p=0.0401$, $p<0.05$) (Table-4).

Comparison of the Scores of the Groups in FST 1 and FST 2

Both groups displayed significantly more duration of immobility in FST 2 in comparison to the duration of immobility they exhibited in FST 1 ($t=5.507$ $df=7$, $p<0,05$ for experimental group and $t=2.717$ $df=7$, $p<0.05$ for the control group (Table-5).

Insert Figure-5 About Here

Wilcoxon Matched-Pairs Signed-Ranks Test indicated that the experimental group performed less diving behavior in FST 2 than in FST 1 ($z=-0.2404$ 2-tailed $p=0.0251$, $p<0.05$) (Table-6a).

Insert Figure-6 About Here

On the other hand, the control group exhibited significantly less jumping and headshaking behaviors in FST 2 in comparison to its jumping and headshaking behavior scores in FST 1 ($z=-2.1129$ 2-tailed significance=0.0346, $p<0.05$ for the jumping behavior and $z=-2.2014$ 2-tailed sig.=0.0277, $p<0.05$ for the headshaking behavior (Table-6b).

Insert Figure-7 and Figure-8 About Here

Discussion

Results of the second experiment indicated an antidepressant-like effect of keeping subjects under D:D condition for 24 hours observed as shorter immobility and increased headshaking behavior in the second FST in comparison to the control group. The present study showed that the two opposite treatments used in our two experiments have similar antidepressant-like effects in the FST; keeping subjects under D:D conditions for 24 hours showed antidepressant-like effects in the FST when subjects were tested in the FST 24 hours later following offset of the treatment.

The present study is the first in the literature showing that converting a 12L:12D light-dark cycle into 12D:12D (24 h dark) has antidepressant-like effects in the FST paradigm. Animals were tested 24 hours after the offset of the D:D period as the no-delay experimental group in the first experiment. Additional observations on different time points should be done, as the first experiment, to determine the temporal limitations of the antidepressant-like efficiency of this treatment. By virtue of this kind of an additional behavioral study it might be possible to compare the antidepressant-like efficiency of these two opposite treatments on the basis of their temporal parameters.

GENERAL DISCUSSION

Results of the first experiment indicated that one session of 12 h light pulse during the dark phase of the L:D cycle has protective antidepressant-like effects in the FST when tested even 7 days after the offset of light exposure. Both the one-day delay experimental and the 7-day delay experimental groups were not significantly more immobile in FST 2 than they were in FST 1. This was an indicator of protection against behavioral despair. Furthermore, the one-day delay experimental group was significantly less immobile in FST 2 than one-day delay control, 4-day delay control and no treatment groups. The 7-day delay group was also significantly less immobile in FST 2 than the one-day delay control and no treatment groups. These experimental groups also did not display significantly less diving and jumping behavior in FST 2 than they displayed in FST 1. However, the 4-day delay experimental group was significantly more immobile in FST 2 than it was in FST-1 and displayed a significant decrease in diving behavior in FST 2 in comparison to its diving score in FST 1. Given the fact that there was no significant difference in any of the parameters among the groups in FST 1, results of FST 2 cannot be a consequence of motor problems of the subjects in any of the control groups or the 4-day delay experimental group.

With regard to the previous work done in our laboratory (Yılmaz et al, 2000), antidepressant-like effects of 12 hours of light exposure was shown to extend to 36 hours after the offset of the light exposure. It seems according to the results of our first experiment that protective efficiency of a single 12 h light pulse during the dark phase of the L:D cycle extends beyond this time point and can be observed 7 days after the offset of a single 12 h light exposure.

As mentioned earlier, the FST is especially sensitive to tricyclic agents and tricyclic agents exert their antidepressant effects by acting on both noradrenergic (NA) and serotonergic systems (5-HT) (Detke, Rickles and Lucki, 1995). Tricyclic antidepressants

such as desipramine protect subjects from behavioral despair in the FST by inhibiting the re-uptake of NA into the presynaptic neuron (Reneric, Bouvard and Stinus, 2001). In the FST, increased 5-HT turnover results in increased escape-oriented behaviors like diving and jumping, while increased turnover of NA is characterised by decreased immobility levels (Detke, Rickles and Lucki, 1995). It is known that administration of specific serotonin re-uptake inhibitors (SSRI's) such as paroxetine fails to protect subjects from behavioral despair in the FST indicating NA system as the specific neurochemical mechanism underlying duration of immobility in the FST (Kennaway et al, 2001).

Pharmacological data indicate that light exposure and tricyclic agents induce similar alterations in circadian rhythms and exert common antidepressant-like effects, especially when the antidepressant treatment is successful (Hill, 1992). Animal studies comparing the efficiency of light exposure with tricyclic antidepressants in the FST-induced behavioral despair paradigm indicate a similarity between the antidepressant efficiency of these agents. As it was shown in the studies of Hernandez et al (1999) and Arushanian (1999), light exposure for two weeks protects male Wistar rats from behavioral despair in the FST as effective as tricyclic antidepressants such as imipramine. In these studies light exposed subjects were not significantly more immobile in FST 2 than they were in FST 1. As reduced immobility levels in the FST is considered as a consequence of increased NA turnover, two weeks of light exposure possibly induced an increase in NA turnover, which protected light exposed subjects from behavioral depression in terms of not being significantly more immobile in FST 2 than they were in FST 1. Two weeks of light treatment was also shown to result in a similar antidepressant-like protection in escape-oriented behaviors, which depends on 5-HT system, in the FST (Arushanian, 1999). Neurochemical studies on effects of light exposure indicates higher amounts of NA and 5-HT metabolites in both humans and animals (Arushanian, 1999; Madden et al 1996; Wirz-Justice et al, 1993). Neuroanatomical studies indicate reciprocal projections between the

SCN and the raphe nuclei, which is the major 5-HT turnover site in the CNS (Kennaway et al, 2000). It is known that the SCN receives serotonergic input especially from the medial and dorsal raphe nuclei and lesions to these regions are shown to desynchronise circadian rhythms by decreasing 5-HT content in the SCN (Kenneway and Moyer, 1998; Kenneway et al, 2000). Light exposure (as brief as 2sec) and antidepressant drug administration are also shown to supress melatonin secretion both in human and animal studies during antidepressant treatment (Kennaway et al,2000; Wirz-Justice et al, 1993).

Given the specific neurochemical mechanisms underlying different behavioral parameters in the FST, results of the first experiment correspond to a tricyclic antidepressant-like effect of light exposure. NA and 5-HT turnover in the subjects in the one-day delay and 7-day delay experimental groups might have been increased due to light exposure, since immobility duration of these experimental groups were not significantly higher and escape oriented behavior (diving and jumping) scores were not significantly lower in FST 2 than they were in FST 1. Furthermore, although there was no significant difference among the groups in FST 1, in FST 2 one-day delay experimental group was significantly less immobile than its own control group and both the one-day delay experimental and 7-day delay experimental groups were significantly less immobile than the no treatment group. Results of the first experiment, along with the pharmacological data on the neurochemical substrates of FST-induced behavioral despair, correspond to increased NA and 5-HT turnover in the one-day delay and 7-day delay experimental groups. However, it is likely that such an increase in NA and 5-HT turnover of the subjects in the 4-day delay experimental group did not occurre. A neurochemical analysis for detecting the amount of NA and 5-HT metabolites in our subjects could provide us with the opportunity of testing this probability.

In addition to the neurotransmitter mechanisms discussed above that are likely to be neurochemical substrates of the present results, neuropeptidergic projections of the SCN to

limbic structures might play a role in the results of first experiment. Photic input is conveyed via the retinohypothalamic tract to the SCN and the SCN are rich in neurohormones and neuropeptides such as vasopressin and corticotropin releasing factor (CRF) that play important roles in behavioral depression paradigms including the FST (Reghunandanan, Reghunandanan and Singh, 1993). The SCN make neuropeptidergic projections to limbic structures such as the amygdala (Onaka and Yagi, 1998; Reghunandanan, Reghunandanan, and Singh, 1993; Rusak and Bina, 1990). Among the limbic structures receiving vasopressin, oxytocin and CRF input from the SCN is the bed nucleus of the stria terminalis (BNST) (van Esseveldt, Lehman and Boer, 2000). Recent research indicate the BNST as the main limbic structure mediating responses under unconditioned fear situations such as the FST and lesions to the BNST are shown to result in behavioral despair in the FST (Schulz and Canbeyli, 2000). It is known that decrease in vasopressin level in the BNST, which also receives the densest NA projections in the CNS, results in a increase in oxytocin level that in turn leads to fear responses (Onaka and Yagi, 1998). There is the probability that light exposure, along with increased turnover of NA and 5-HT, might induce alterations in neuropeptide levels in limbic structures such as the BNST via neuropeptidergic projections from the SCN to those limbic structures.

In addition to the neurochemical and neurophysiological mechanisms discussed above, phase change, particularly phase delay, of the rhythms may be considered as a main indicator and factor underlying antidepressant efficiency of antidepressant treatments (Detke, Rickles and Lucki, 1995; Stewart et al, 1990b). However, phase delay of the rhythms might not be a necessity for alleviating depressive symptoms and/or protecting from depression due to a subsequently applied behavioral depression paradigm. There is not always a positive correlation between phase delay and alleviation of depression in the literature; phase advances and lengthening of free running period may well serve the same function. For example in a study by Stewart et al (1990a) it was shown that animals, in

which a period lengthening effect was observed by virtue of exposure to different L:D cycles, performed significantly better in a shuttle-escape task in comparison to animals displaying entrainment or short free running period. In another study by the same authors (Stewart et al, 1990b), inescapable electric shocks were delivered to subjects in a shuttle-box in an escape-learning setting. Daily activity rhythms of the subjects were assessed via activity wheels for determining delays or advances in these rhythms and it was found that both lengthening and shortening in the free running periods of the subjects were positively correlated with escape-learning performance in the shuttle-box. Pharmacological data are also in line with this conclusion in that chronic administration of lithium and many of the antidepressant drugs are known to induce lengthening in free running period in both humans and rats (Heiser and Wilcox, 1998; Redrobe and Bourin, 1999). It is proposed that alterations in circadian rhythms, both phase advances and delays, probably along with stress-related hormonal mechanisms, are the components of a common intrinsic antidepressant mechanism providing the organism with the opportunity for optimal adaptation to stressful environmental conditions (Stewart et al, 1990b).

Converting the L:D cycle to either L:L or D:D, similar to the treatment in Stewart et al (1990a), can modulate free running period (Pittendrigh, 1988). It is possible that in our study changing 12L:12D cycle to 12L:12L for 24 hours by 12 h light pulse exposed during the subjective night (19:00-07:00) altered free running periods of the subjects, which were protected from behavioral despair in the FST.

The finding that, a 12 h light exposure during the dark phase of the L:D cycle for one night can protect from behavioral despair in the FST when tested 7 days after the offset of the treatment is the first in the literature. The next step for future research should be to assess the changes in circadian rhythm (activity rhythms for instance) of each subject individually to find the circadian basis of the observed antidepressant-like protective effects of a single 12 h light pulse. If free running circadian activity rhythms are assessed

for each of our subjects in a future research, it will be possible to depict a general pattern and direction of alteration in the circadian rhythms of animals, which are protected from behavioral despair in the FST.

Results of the second experiment, which placed rats on a temporary D:D cycle, indicated that exposing subjects to continuous darkness for 24 hours (changing 12L:12D cycle to 12D:12D) results in decreased time spent immobile and increased headshaking behavior in comparison to the control group. While it has been known for some time that continuous darkness has some protective effects on the metabolism through melatonin secretion (Rusak and Bina, 1990), the second experiment is the first study in the literature that showed antidepressant-like effects of this treatment in the FST carried out under diurnal conditions.

It is known that when behavioral tests such as FST are carried out under nocturnal conditions, animals behave less stressed in comparison to diurnal conditions (Kelliher et al, 2000). In the pharmacological literature this situation is considered to be a consequence of activation of neurohormones such as corticosterone and pineal melatonin, which is continuously secreted from the pineal gland during dark period (Abel, 1993; Shaji and Kulkarni, 1998). Even in nocturnal organisms, including rodents, melatonin turnover is highest at nights (Kelliher et. al, 2000). Melatonin receptors are widely distributed in the CNS comprising important regions such as the amygdala (especially in the central nucleus), hypothalamus and the SCN, which are the key structures underlying depression and anxiety (Shaji and Kulkarni, 1998). In fact, melatonin is shown to have both antidepressant and anxiolytic properties, which reduces neophobic responses. (Eison et al, 1995; Kopp et al, 1999).

It is likely that converting L:D cycle to D:D for the experimental group for a period of 24 hours might have resulted in continuous secretion of pineal melatonin during the entire dark phase, which could also lead to an increase in NA and 5-HT levels (Shaji and

Kulkarni, 1998). However, it has not been studied whether elongated secretion of pineal melatonin due to continuous darkness, which might be the neurochemical process underlying present results, exerts antidepressant-like properties in the FST when carried out under diurnal conditions. This question might be answered by measuring and comparing the melatonin level in the experiment and control subjects in an additional experiment.

At first glance, it seems paradoxical on a neurochemical basis that both L:L and D:D treatments have antidepressant-like efficiency in the FST, since light exposure suppresses melatonin synthesis in the pineal gland, on the other hand dark exposure leads to an increase in the synthesis of melatonin. However, pharmacological data indicate that melatonin enhances NA and 5-HT turnover and plasma melatonin level is positively correlated with the NA level (Feenstra et al,2000). Due to this property of melatonin, L:L and D:D treatments are likely to lead to convergent neurochemical processes; light exposure increases 5-HT and NA turnover along with suppressing melatonin synthesis in the pineal gland and dark exposure probably increases the NA and 5-HT turnover as a consequence of increased melatonin level.

Another possibility is that D:D treatment in the second experiment might have induced sleep deprivation in the experimental subjects. Rats, like other rodents, are nocturnal organisms and are active at night (Kelliher et al, 2000). Keeping our experimental subjects under 24 hours of continuous darkness could have induced a kind of sleep deprivation or low-quality sleep in these animals.

Sleep deprivation is being used as an antidepressant therapeutic method, but it is well established in the literature that antidepressant effects of sleep deprivation are quite transient and diminish when subjects are allowed to sleep after the deprivation period (Wehr and Wirz-Justice, 1982). Animal studies indicate that, 12 hours of sleep deprivation ameliorates the performance of depressed rats in the open field test after exposition to

social defeat paradigm (Szuba et al, 1994). There is evidence in the literature about the potentiation of antidepressant-like effects of sleep deprivation when combined with serotonergic drugs (Wehr and Wirz-Justice, 1982). Psychopharmacologic agents like lithium, clomipramine and fluoxetine are known to support the antidepressant effect of sleep deprivation and they are able to prevent relapse after subsequent sleep (Szuba et al, 1994).

On a circadian basis, it likely that living under a constant L:D cycle for 24 hours, probably in a similar way to the first experiment, modulated (shortened or lengthened) circadian periods of our experimental subjects, which in turn led to a lower duration of immobility in FST 2 when compared to control subjects. Assessing the circadian rhythm alterations of each experimental subject could give us the opportunity to test this probability and, if so, determine what percent of our subjects displayed period lengthening and what percent of them displayed period shortening.

The present experiments showed that modulating 12L:12D cycle in either 12L:12L or 12D:12D direction for 24 hours has antidepressant-like protective effects on the FST-induced behavioral despair paradigm. Our data also indicate that D:D treatment might not be as effective as L:L treatment in protecting from FST-induced behavioral despair, since in the second experiment, unlike the one-day delay and 7-day delay experimental groups of the first experiment, the experimental group was more immobile in FST 2 than its own duration of immobility in the FST 1.

However, at this point it is not possible to compare these two treatments, directly, on the temporal basis of their antidepressant-like efficiency. The second experiment is a pioneer in the literature, which indicated that exposing subjects to D:D condition for 24 hours has antidepressant-like effects on FST-induced behavioral despair when tested 24 hours after the offset of the D:D condition. Further studies targeting such a comparison should be conducted. For instance, an additional study may be carried out to test the

subjects on different time points in FST beyond the 24 hours. Such a study could provide us an opportunity to compare antidepressant-like efficiency of these two treatments, at least, on the basis of temporal parameters.

Such an additional study to our second experiment should also comprise the assessment of individual alterations in the rhythms of the subjects. This kind of an assessment can show us both whether antidepressant-like protective effects of D:D condition for 24 hours can be correlated with any apparent alteration in the circadian rhythms of the subjects and, if so, the general pattern of this alteration.

In conclusion, results of the present experiments, which indicated that converting 12L:12D cycle to both 12L:12L or 12D:12D condition for 24 hours leads to similar antidepressant-like effects, are the first findings in the literature. It is observed that changing the L:D cycle for 24 hours via 12 h light or dark pulse is potent enough to protect from the FST-induced learned despair even 7 days after the offset of the treatment as shown in the first experiment. Further research is needed to explore the circadian and neurochemical processes underlying the antidepressant-like protective efficiency of these two opposite L:D cycles on the FST-induced behavioral despair paradigm.

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FIGURES

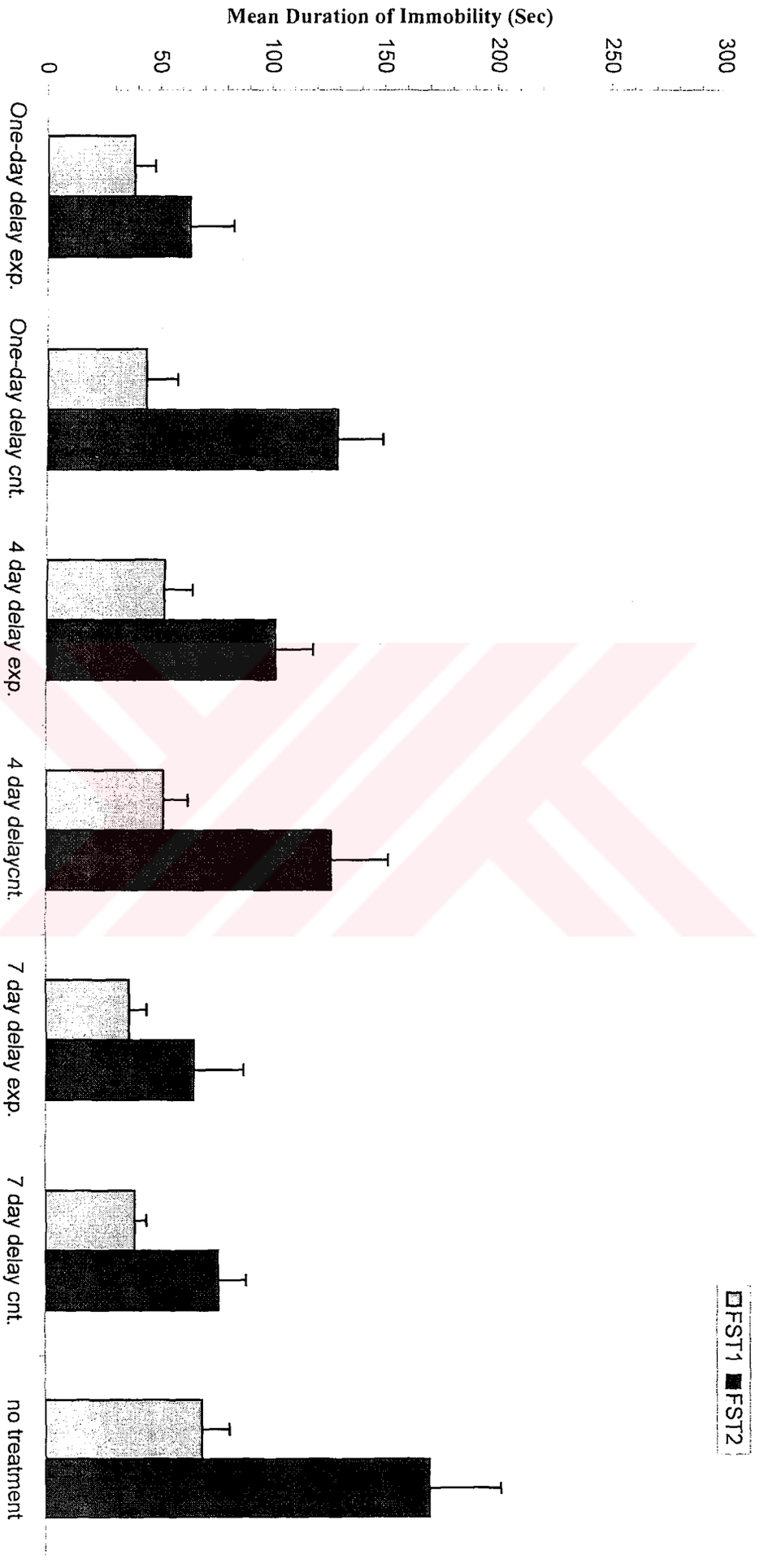

Fig.1-Changes in the mean duration of immobility of the groups across FST-1 and FST-2.Error bars show standard error of mean.

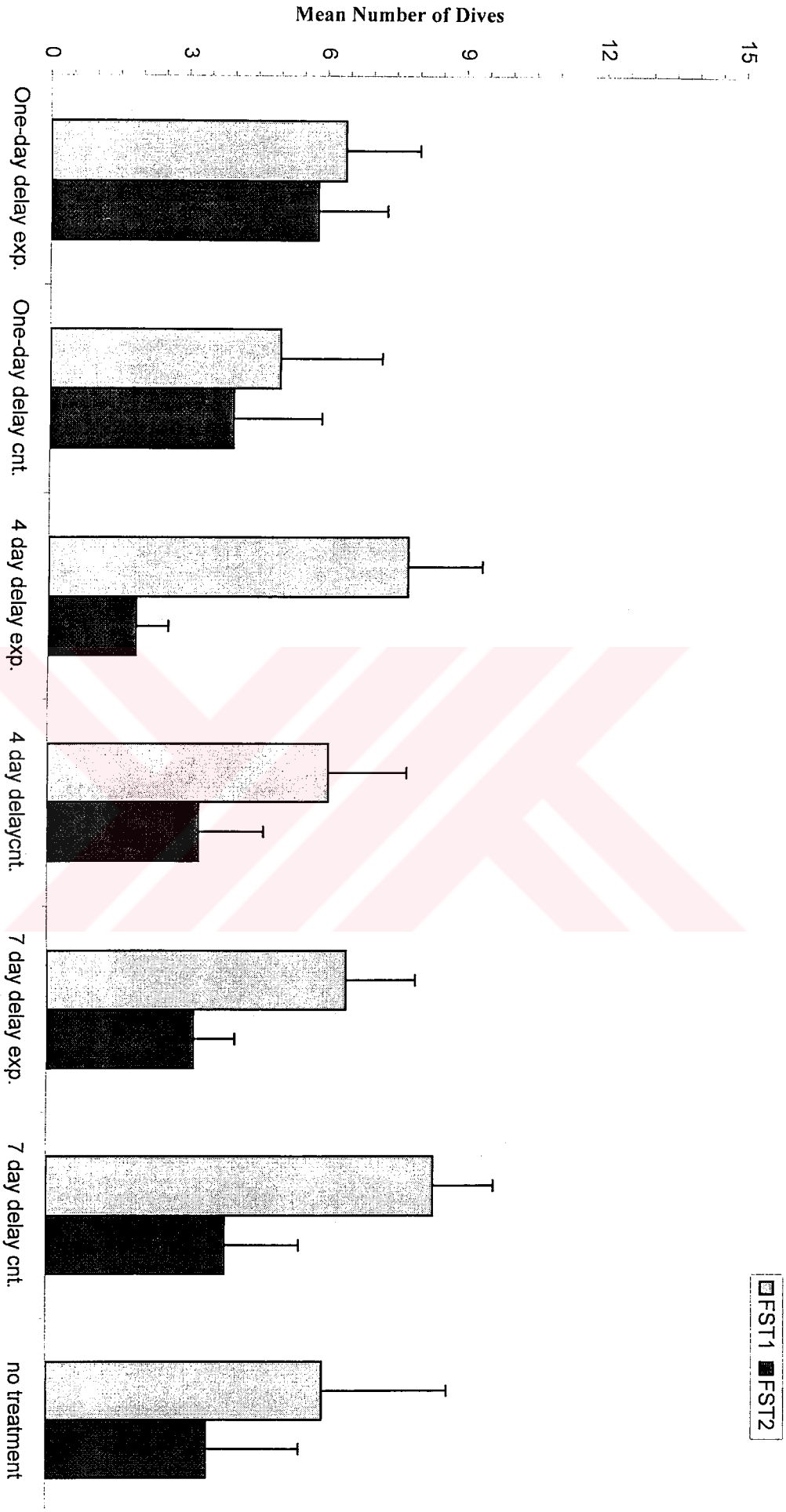

Fig.2- Changes in mean diving behavior scores of the groups across FST1 and FST2.Error bars show standard error of mean.

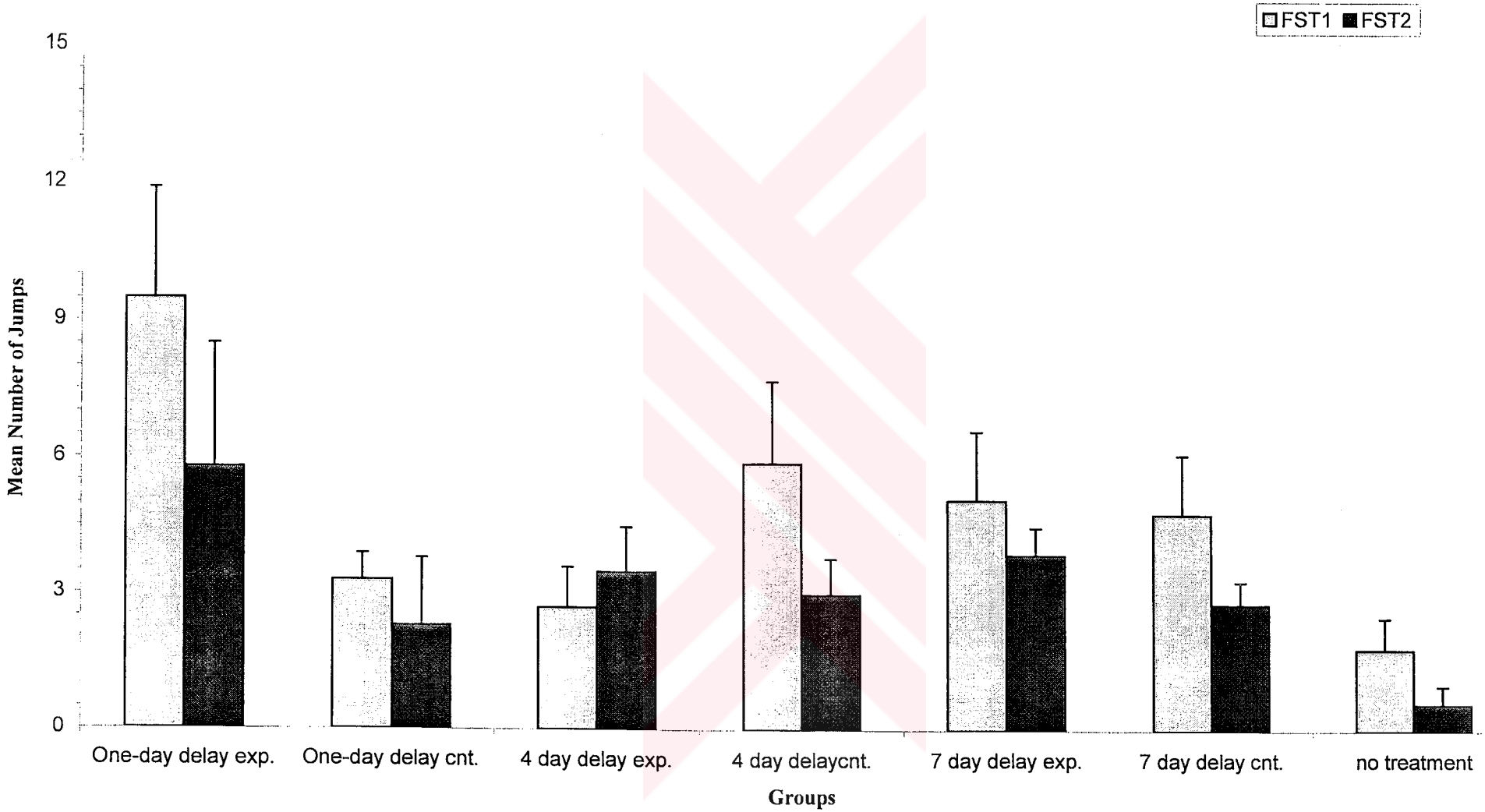

Fig.3-Changes in mean jumping behavior scores of the groups across FST1 and FST2. Error bars show standard error of mean.

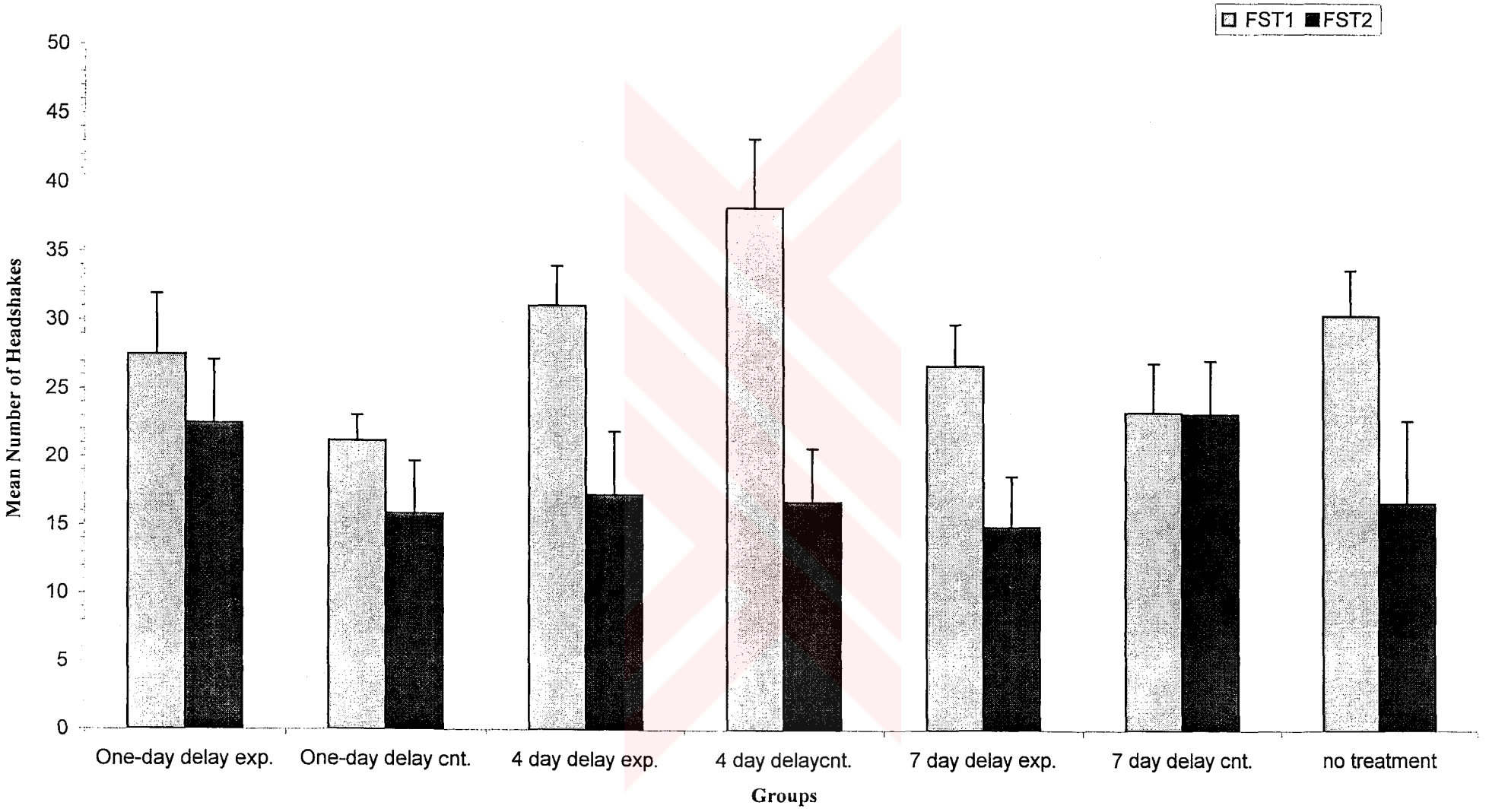

Fig.4-Changes in mean headhaking behavior scores of the groups across FST1 and FST2.Error bars show standard error of mean.

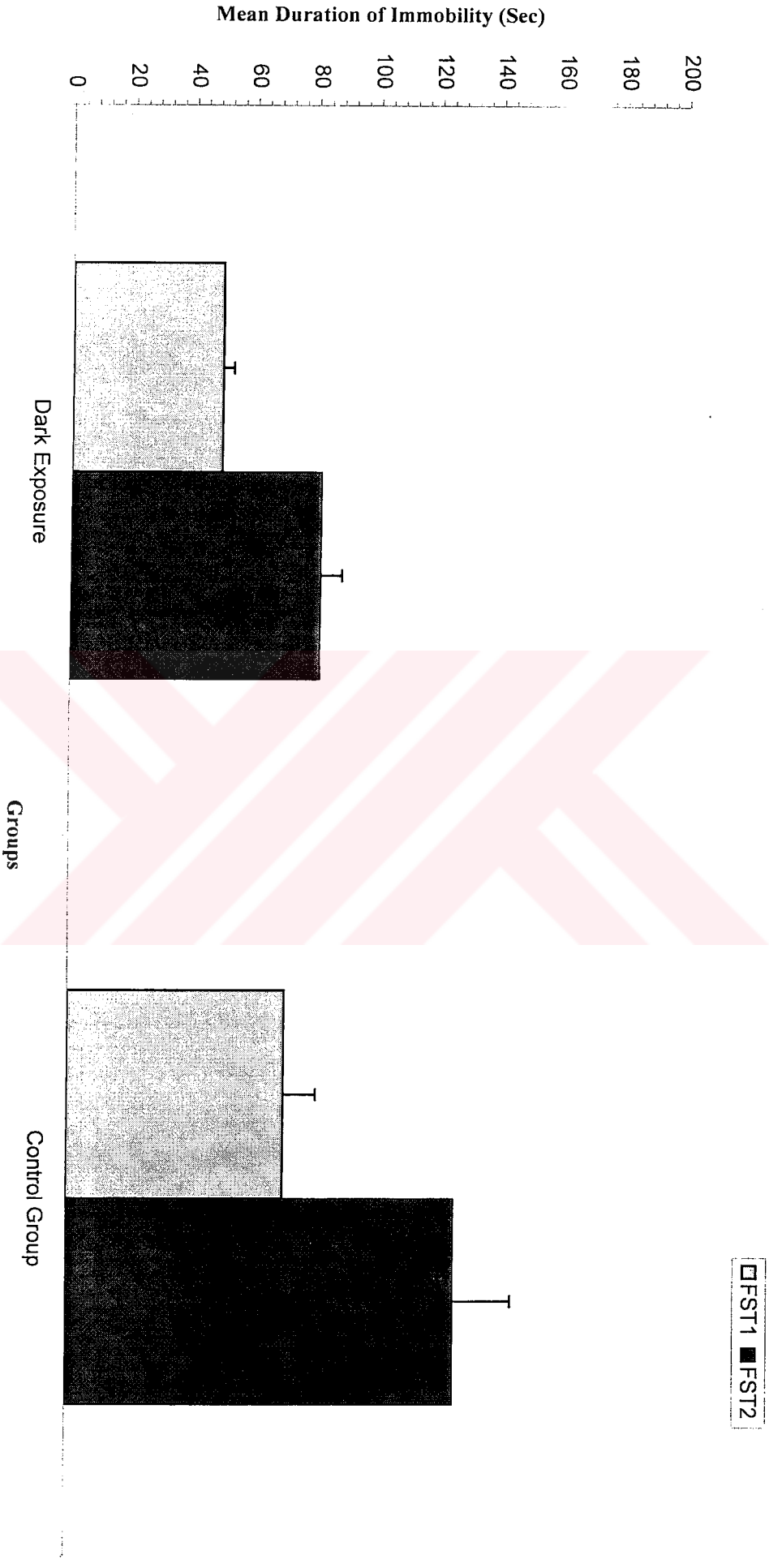

Fig.5-Changes in mean duration of immobility of the groups across FST 1 and FST 2. Error bars show standard error of mean.

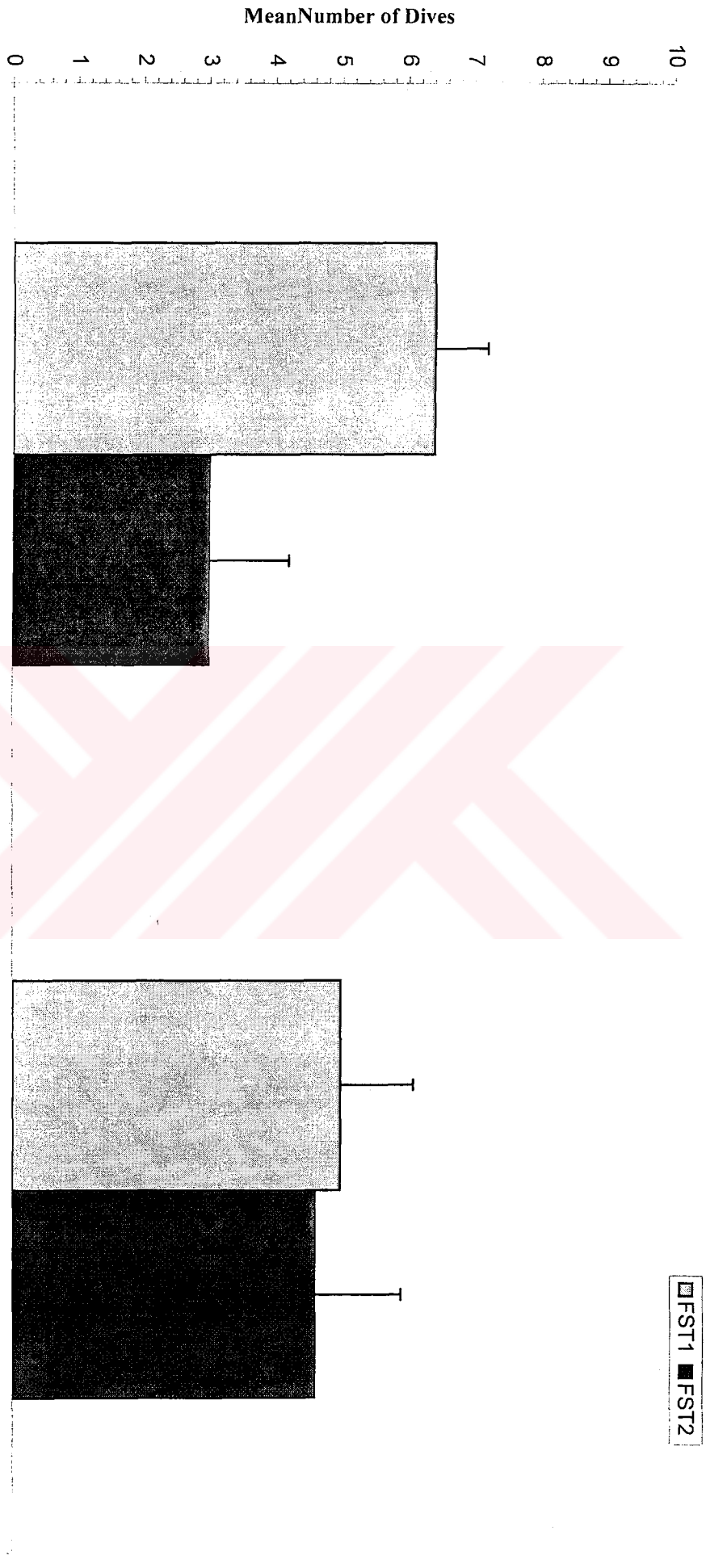

Fig.6-Changes in the mean diving behavior scores of the groups across FST1 and FST2.Error bars show standard error of mean.

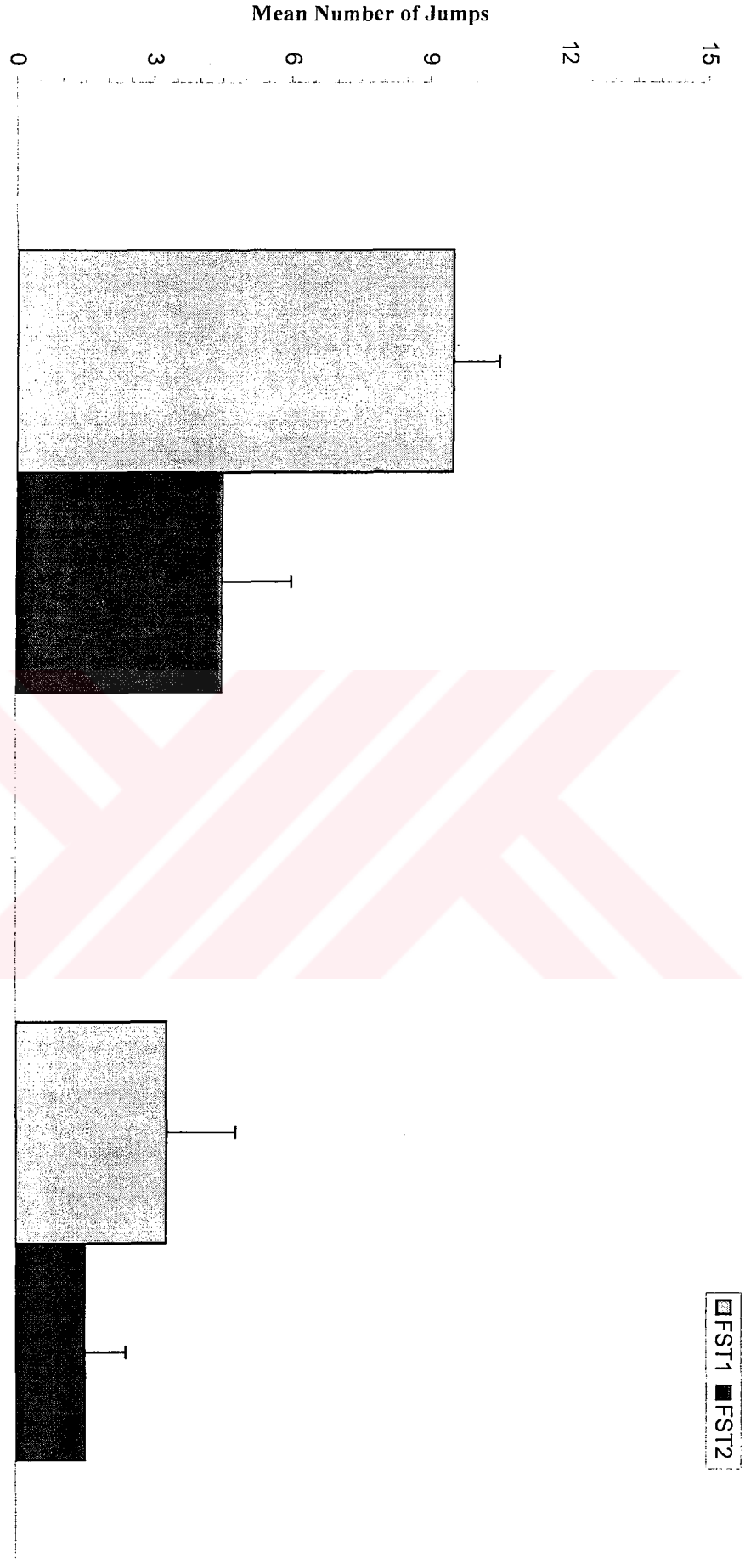

Fig.7-Changes in the mean jumping behavior scores of the groups in FST1 and FST2. Error bars show standar error of mean.

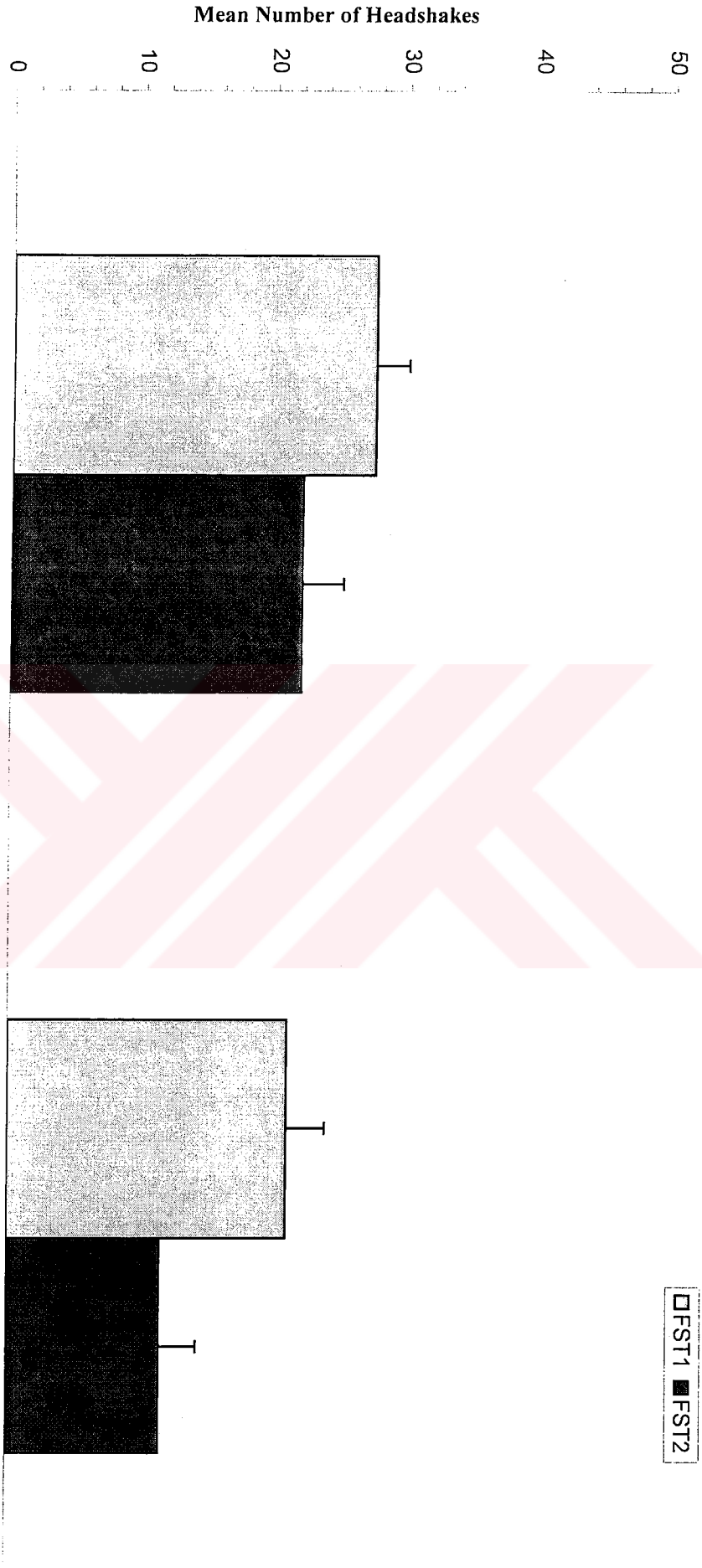

Fig.8-Changes in mean headshaking behavior scores of the groups across FST1 and FST2.Error bars show standard error of mean.

APPENDICES

Appendix 1: Tables of the Experiment 1

Table-1a) Means and standart deviations of the groups in FST 1

	Duration of Immobility	Diving	Jumping	Headshaking
Groups	**Mean ± Std**	**Mean ± Std**	**Mean ± Std**	**Mean ± Std**
One- day delay Exp.	38.4 ± 26.6	6.4 ± 4.6	9.5 ± 6.7	27.5 ± 12.5
One-day delay Cnt.	43.8 ± 39.4	5.0 ± 6,3	3.3 ± 1.5	21.2 ± 5.4
4-Day Delay Exp.	52.3 ± 36.1	7.8 ± 4.6	2.7 ± 2.5	31.1 ± 8.3
4-Day Delay Cnt.	52.0 ± 31.4	6.1 ± 4.9	5.9 ± 5.1	38.3 ± 14.3
7-Day Delay Exp.	29.0 ± 21.9	6.5 ± 4,3	5.1 ± 4.2	26.8 ± 8.4
7-Day Delay Cnt.	39.4 ± 15.3	8.4 ± 3.5	4.8 ± 3.6	23.4 ± 10.3
No-treatment	69.8 ± 34.7	6.0 ± 7.7	1.8 ± 2.2	30.5 ± 9.3

Table-1b) Means and standart deviations of the groups in FST 2

	Duration of Immobility	Diving	Jumping	Headshaking
Groups	**Mean ± Std**	**Mean ± Std**	**Mean ± Std**	**Mean+ Std**
One-day delay Exp.	63.5 ± 54.9	5.8 ± 4.3	5.8 ± 7.5	22.5 ± 12.3
One-day delay Cnt.	129.4± 57.5	4.0 ± 5.4	2.3 ± 4.1	15.9 ± 10.8
4-Day Delay Exp.	102.0± 46.1	1.9 ± 2,1	3.5 ± 2.9	17.3 ± 12.9
4-Day Delay Cnt.	127.1± 71.4	3.3 ± 3.4	3.0 ± 2.2	16.8 ± 11.2
7-Day Delay Exp.	66.1 ± 62,0	2.9 ± 2.3	2.4 ± 1.8	18.0 ± 10.5
7-Day Delay Cnt.	77.1 ± 34.7	3.9 ± 3.3	3.9 ± 1.6	20.3 ± 11.0
No-treatment	171.1± 89.7	3.5 ± 5.7	0.6 ± 1.2	16.8 ± 17.2

Table-2a) One-Way ANOVA table of duration of immobility of the groups in FST 1

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	8274.61	6	1379.101	1.494	.200
Within Groups	45240.3	49	923.270		
Total	53514.9	55			

F(6,49)=1.769 P>0.05

Table-2b) Kruskall-Wallis One-Way ANOVA table of diving, jumping and headshaking behaviors of the groups in FST 1

Behaviors	Chi-Square	D.F.	Significance
Diving in FST 1	4.482	6	0.612
Jumping in FST 1	11.295	6	0.800
Headshaking in FST 1	11.240	6	0.810

Table-3a) One-Way ANOVA table of duration of immobility of the groups in FST 2

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	75803.464	6	12633.911	3.310	.008
Within Groups	187051.375	49	3817.375		
Total	262854.839	55			

F(6,49)=3.310, P<0.05

Table-3b) LSD results: Significant differences in duration of immobility among the groups in FST 2

Groups	Mean Difference	Std.Error	Significance
One-day delay exp - Oneday delay Cnt.	-65.875	30.8295	0,038
One-day delay exp - 4- Day Delay Cnt.	-63.125	30.8295	0.045
One-day delay exp-No-treatment	-107.625	30.8295	0.001
One-day delay Cnt-7- Day Delay Exp.	-63.25	30.8295	0.046
4-Day Delay Exp-No-treatment	-69.125	30.8295	0.03
7-Day Delay Exp-No-treatment	-105	30.8295	0.001
7-Day Delay Cnt-No-treatment	-94	30.8295	0.004

Table3) Kruskall-Wallis One-Way ANOVA table of diving, jumping and headshaking behaviors of the groups in FST 2

Behaviors	Chi-Square	D.F.	Significance
Diving-FST 2	4.283	6	0.638
Jumping FST 2	11.365	6	0.780
Headshaking FST 2	3.250	6	0.777

Table 4) One-Samle t-Test results of the comparison of each group's duration of immobility in FST-2 with its own duration of immobility in FST 1

Groups	t	df	Sig. (2-tailed)	Mean Difference
One-day delay experiment	1.296	7	0.236	25.1250
One- day delay control	4.212	7	0.004	38.6250
4-Day delay experiment	2.997	7	0.020	49.7500
4-Day delay control	2.975	7	0.021	75.1250
7-Day delay experiment	1.692	7	0.134	37.1250
7-Day delay control	3.048	7	0,019	37.3750
No-treatment	5.394	7	0.015	101.3750

Table-6a) Wilcoxon Matched-Pairs Signed-Ranks Test results of diving behavior in FST 2

Group	Cases	Z-score	Two-Tailed P	Significance
One-day delay experiment	8	-.4226	0.6726	p>0.05
One-day delay control	8	-.6290	0.5294	p>0.05
4-Day delay experiment	8	-2.3664	0.0180	p<0.05
4-Day delay control	8	-1.1832	0.2367	p>0.05
7-Day delay experiment	8	-1.4003	0.1614	p>0.05
7-Day delay control	8	-1.8970	0.0580	p>0.05
No-treatment	8	-.9435	0.3454	p>0.05

Table-6b) Wilcoxon Matched-Pairs Signed-Ranks Test results of jumping behavior in FST 2

Groups	Cases	Z-score	Two-Tailed P	Significance
One-day delay experiment	8	-.9802	0.3270	p>0.05
One-day delay control	8	-.4226	0.6726	p>0.05
4-Day delay experiment	8	-.7702	0.4412	p>0.05
4-Day delay control	8	-1.8204	0.0687	p>0.05
7-Day delay experiment	8	-1.6803	0.0929	p>0.05
7-Day delay control	8	-.493	0.6220	p>0.05
No-treatment	8	-1.0483	0.2945	p>0.05

Table-6c) Wilcoxon Matched-Pairs Signed-Ranks Test results of headshaking behavior in FST 2

Groups	Cases	Z-score	Two-Tailed P	Significance
One-day delay experiment	8	-1.3303	0.1834	p>0.05
One-day delay control	8	-1.0987	0.2719	p>0.05
4-Day delay experiment	8	-1.8204	0.0687	p>0.05
4-Day delay control	8	-2.5205	0.0117	p<0.05
7-Day delay experiment	8	-2.0284	0.0425	p<0.05
7-Day delay control	8	-.1410	0.8880	p>0.05
No-treatment	8	-1.8204	0.0687	p>0.05

Appendix 2: Tables of the Experiment 2

Table-2a) Means and standard deviations of the groups in FST 1

	Duration of Immobility	Diving	Jumping	Headshaking
Groups	**Mean ± Std**	**Mean ± Std**	**Mean ± Std**	**Mean ± Std**
Experiment Gr.	49.375± 10.013	6.4 ± 2.326	9.5 ± 2.9	27.5 ± 7.21
Control Gr.	70.875 ± 25.083	5.0 ±3.151	3.3 ± 4.207	21.2 ± 8.408

Table-2b) Means and standard deviations of the groups in FST 2

	Duration of Immobility	Diving	Jumping	Headshaking
Groups	**Mean ± Std**	**Mean ± Std**	**Mean ± Std**	**Mean ± Std**
Experiment Gr.	81.625 ± 19.131	3 ± 3.215	4.5 ± 4.209	22.125 ± 9.031
Control Gr.	126.375 ±52.568	4.625±3.777	1.5 ± 2.507	11.75 ± 7.888

Table-2) Independent Samples t-Test results of comparison of immobility duration of the groups in FST 1

T	Df	Significance
-3.4810	14	0.054

Table-3) Mann-Whitney U-Wilcoxon Sum W Test Analysis results of comparison of diving, jumping and headshaking behaviors of the groups in FST 1

Behaviors	U	W	Exact 2-Tailed P	Z	2-Tailed P
Diving	28.5	71.5	0.7209	-0.3757	0.7072
Jumping	22	78	0.3282	-1.0612	0.2886
Headshaking	15	85	0.083	-1.7893	0.0736

Table-4) Independent Samples t-Test results of comparison of immobility duration of the groups in FST 2

F	Significance	T	Df	Significance
6.850	0.020	-2.2650	14	0.040

Table-5) Mann-Whitney U-Wilcoxon Sum W Test Analysis results of comparison of diving, umping and headshaking behavior score means of the groups in FST 2

Behaviors	U	W	Exact 2-Tailed P	Z	2-Tailed P
Diving	24.5	60.5	0.4418	-0.7983	0.4247
Jumping	14.5	85.5	0.065	-1.8989	0.0576
Headshaking	12.5	87.5	0.0379	-2.0524	0.0401

Table6) One-Sample t-Test results of the comparison of immobility duration of the groups in FST-2 with their own duration of immobility in FST 1

Groups	T	Df	Significance	Mean Difference
Experiment Gr.	5.507	7	0.001	37.2500
Control Gr.	2.717	7	0.030	50.000

Table-7a)Wilcoxon Matched-Pairs Signed-Ranks Test results of the comparison of diving, jumping and headshaking behaviors of the experimental group in FST-2 with its own scores in FST 1

Behaviors	Z	2-Tailed P	Sig.
Diving	-0.2404	0.0251	P<0.05
Jumping	-0.4901	0.6241	P>0.05
Headshaking	-1.3303	0.1834	P>0.05

Table-7b) Wilcoxon Matched-Pairs Signed-Ranks Test results of the comparison of diving, jumping and headshaking behaviors of the control group in FST-2 with its own scores in FST 1

Behaviors	Z	2-Tailed P	Sig.
Diving	-2.1129	0.0346	P<0.05
Jumping	-1.278	0.2012	P>0.05
Headshaking	-2.2014	0.0277	P<0.05