# ANATOMY OF ANTIDEPRESSANT EFFECT OF LIGHT:

# THE ROLE OF THE BNST LESIONS

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2008

# ANATOMY OF ANTIDEPRESSANT EFFECT OF LIGHT: THE ROLE OF THE BNST LESIONS

Thesis submitted to the

Institute for Graduate Studies in the Social Sciences

in partial fulfillment of the requirements for the degree of

Master of Arts

in

Psychology

by

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Boğaziçi University

2008

Thesis Abstract

Billur Avlar, “Anatomy of Antidepressant Effect of Light:

The Role of the BNST Lesions”

The aim of the present study was to shed light on the anatomy of antidepressant effect of bright light therapy. In line with this, lesions of the Bed Nucleus of the Stria Terminalis (BNST) which is implicated as an important relay area in mediating stress related circuitry in the brain, or sham operations were done in male Wistar rats. Nine weeks later, a single 10 min, 200 watt, 1350 lux bright light stimulus was administered at ZT21 (04:00) to lesioned (LL) or sham operated rats (SL). Control group animals; either lesioned (LC) or sham operated (SC) did not receive light stimulus. Behavioral despair was assessed by consecutive forced swim tests. The results replicated the aggravation of the immobility behavior in LC group and demonstrated an ameliorative effect of the light even in the presence of the BNST lesion (LL). These findings suggest that the BNST lesions confined to mostly the medial division do not have an integral role in mediating the antidepressant effects of light.

Tez Özeti

Billur Avlar, “Işığın Antidepresan Etkisinin Anatomisi:

BNST Lezyonlarının Rolü”

Bu çalışmanın amacı ışık terapisi ile gözlenen antidepresan etkinin anatomisine ışık tutabilmektir. Bununla bağlantılı olarak, stres ile ilişkili devrelere etkisi gözlenen,önemli bir geçiş bölgesi olan BNST yapısına lezyonlar yapılmıştır. Deneklerin diğer yarısı ise Sham-kontrol olarak ameliyat edilmiştir. Bu deneyde erkek Wistar sıçanları denek olarak kullanılmıştır. Ameliyatlardan dokuz hafta sonra, ZT21 (04:00) 10 dakika, 200 watt, 1350 lüks parlak ışık lezyonlu (LL) ve lezyonsuz (SL )hayvanlara tatbik edilmiştir. Bunun yansıra lezyonlu (LC ) veya sham (SC) gruplarından iki adet kontrol grubu da ışık almaksızın aynı deneysel işleme tabi tutulmuşlardır. Davranışsal çaresizlik zorunlu yüzme testi ile ölçülmüştür. Sonuçlar LC grubu için hareketsizliğin artış gösterdiğini replike etmiştir ve ayrıca ışığın koruyucu etkisinin BNST bölgesinin lezyonu ile dahi korunduğunu ortaya koymuştur. Bu sonuçlar, özellikle BNST bölgesinin medyal kısımlara yoğunlaşmış lezyonların ışığın antidepresan etkisinde ayrılmaz bir rolü olmadığını göstermektedir.

ACKNOWLEDGEMENTS

Among the other people, first of all, I would like to thank Prof. Dr. Reşit Canbeyli for injecting the sweetest poison in my mind, the neuroscience. Starting with the PSY 271 course, he became a master source of all information regarding neuroscience for me; I could not reach my current understanding of science and to the pursuit of perfection without his existence.

I am grateful to the encouragement and guidance of Assist Prof. Güven Güzeldere. His efforts and attention from overly distances helped a lot. His sense of humanity made some of the things more bearable for me.

I would like to thank Assist. Prof. Serra Müderrisoğlu for her support during my undergraduate and graduate years. Her influence as training me as a mentor had a significant impact in my life path.

I would like to thank Prof Dr. Falih Köksal for his understanding and support during the preparation of my thesis.

I would like thank all the people and assistants from Psychobiology Laboratory who helped during the preparation of this thesis, the amount of work and their efforts were very valuable for me.

I would like to dedicate my thesis to my family; they are the greatest support and the real believers for me in all kind of situations. Their love and endless belief in my abilities and ambition gave me the strength to carry on and to be persistent in my way. I would like thank my father who always believed in me and has an undeniable effect on my debate ability. I would like to thank to my sister, who acted as she was the biggest sister for me and always be there when I was frustrated and need counseling. I would like to give a special emphasis to my mother, her support and way of understanding the education and her vision which is beyond the time and place that she was captured.

The process of the preparation of my thesis was more effortful than I had ever expected. Most importantly, I will regard this work as an indication of my willingness and ambition to pursue my enthusiasm and never ending curiosity for the science and particularly the mechanism of brain.

Now, I believe, my angels never left me during the hardest times.

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# CHAPTER ONE

## INTRODUCTION

Major Depression is a psychological disorder that has high incidence rates in the populations of diverse countries. Unipolar depression ranked fourth in leading causes of premature mortality and disability (Murray and Lopez, 1997).

Increase in stress-related pathologies in humans has led researchers to concentrate their investigations on different types of therapeutic solutions. Use of different varieties of antidepressant drugs or electroconvulsive therapy (ECT) has not necessarily provided an acceptable solution for many patients (Terman, 2007). Divergence in the responsiveness of the individuals to various medications used in major depression reveals the complexity of this abnormality; interaction of the individual canvas of the organism and the mechanisms that are implicated in stress pathology (Heimer, 2003). Thus, additional therapeutic lines lead us to the search of alternative or adjuvant treatments (Terman, 2007; Terman et al., 2001).

Efficacy of bright light therapy in seasonal affective disorder is confirmed by several studies (Rosenthal et al., 1984; Westrin and Lam, 2007; Wirz-Justice et al., 1993). Addition of bright light therapy as a new antidepressant in treatment of non seasonal depression types is recently being considered; ameliorative effects of bright light therapy in the non-seasonal depression types in humans is established (Even et al., 2008; Kripke, 1998, Kripke et al.1992). Partonen and Lönnqvist (2000) had shown the alleviating role of the bright light exposure in healthy indoor workers during winter time. Despite the fact that there are some conflicting findings regarding the timing of the bright light stimulus (Yamada et al., 1995) a great deal of evidence

has been collected about the antidepressant effect of light treatment in the morning (Kripke et al., 1992; Terman, 2007). As one of the symptoms of major depression-the psychomotor retardation (Katz et al., 2004), is seen in the mornings commonly, alerting quality of the bright light gains an important part in the depression pathology (Cajochen, 2007). Moreover, the strength of the bright light therapy is its rapid onset of antidepressant benefit (Kripke, 1998).

The precise mechanism underlying the antidepressant effect of light treatment has not been fully elucidated, but results from several investigations supports the therapeutic value of circadian phase adjustment (Benedetti et al., 2007). Due to ethical considerations about human studies, animal studies are potent sources for illumination of these mechanisms. However, there are only a handful studies on animals regarding the therapeutic role of the bright light therapy (Molina-Hernandez and Tellez-Alcantra, 2000; Prendergast and Nelson, 2005; Schulz et al., 2008; Yılmaz et al., 2004).

Behavioral despair, using forced swim tests, a well established model of depression was designed to assess the induced depression in animals, especially in rodents (Connor et al., 2000; Connor et al., 1997; Porsolt, LePichon, and Jalfre, 1977; Porsolt et al., 1978). Forced Swim Tests (FSTs) consist of two consecutive days of testing. The duration of the swimming on the first day is 15 minutes and 5 minutes in the second day. The first five minutes of both days are measured and the increase in the immobility scores in the second day is used to predict behavioral despair. The validity of the test was confirmed by the shortening of the duration in immobility scores of the second day after the use of antidepressants and by light exposure used as phototherapy (Molina-Hernandez and Tellez-Alcantara, 2000, Yılmaz et al., 2004).

Detke et al. (1995) introduced a new method to study the different behaviors of the rat during the forced swimming tests. These parameters consist of immobility- defined by the small movements and paw actions to keep the head up from the water, swimming- active swimming including the continuous transitions between the quadrants with wide paw actions and diving, climbing- including the rigorous struggle behavior in terms of the jumps in which nearly the half of the body of the rat comes out of the water generally up against the walls of the cylinder. Frequencies of these behaviors are recorded in five second intervals throughout the forced swim tests and the most dominant pattern of the behavior is coded for each 5-sec interval. This new method in evaluating the FSTs enables a clearer distinction among the behaviors that can be observed throughout the test with a more efficient way of assessing the relation between the new line of antidepressants and the corresponding behavior patterns in the FST (Cryan et al. 2002) and thus is more useful in the investigation of the structural representations in the brain corresponding to these behaviors.

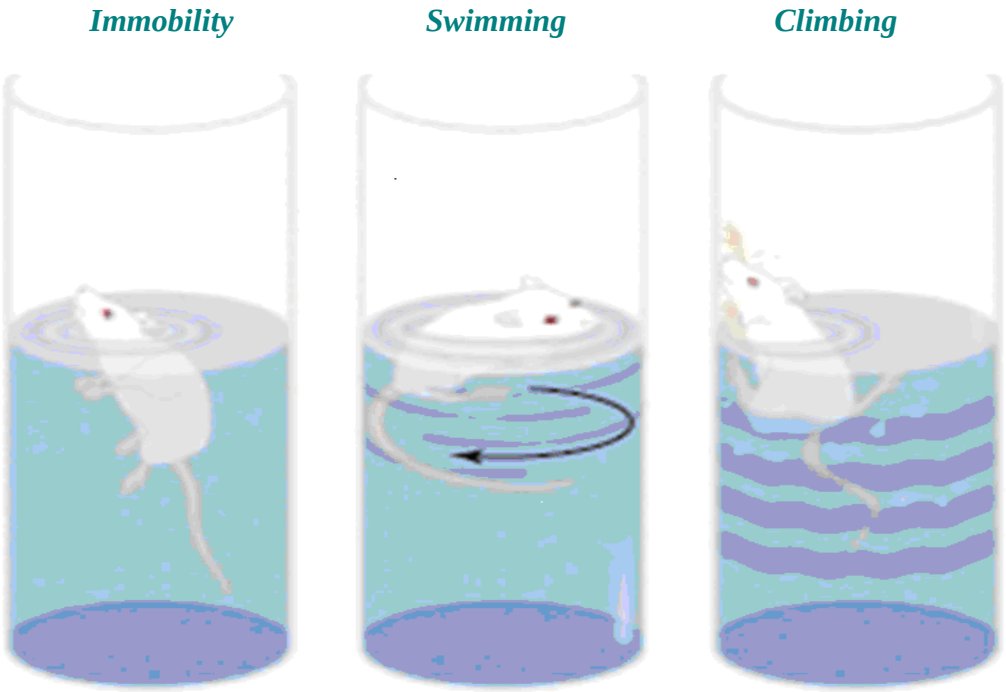



Fig.1: Demonstration of the behavior patterns in the modified FST, adapted from Cryan and Lucki, 2000).

The master pacemaker, the suprachiasmatic nucleus of the hypothalamus (SCN) has a distinguishing role in regulating endocrine and behavioral rhythms (Turek 2001). Zeitgeber (time giver) is a term for denoting the timing of the external cues for entrainment of the endogeneous rhythm of the organism. Light is the most important Zeitgeber for the entrainment of the SCN. ZT 12 (Zeitgeber 12) denotes the subjective beginning of the dark period and ZT0 stands for the light onset when animals are kept on a 12 h light: 12 h dark lighting schedule.

Brain circuits and the structures that are implicated in stress psychopathology show robust circadian rhythmicity. Therefore, inquiry into the role of the circadian functioning in the etiology of the mood disorders is becoming more plausible. Common symptoms that are observed in the depression pathology such as, eating and sleep irregularities require circadian synchronization among the areas in intact brain (Benedetti et al., 2007; Even et al., 2008). In line with the involvement circadian

functioning in the depression pathology, the SCN lesions were shown to have an ameliorative effect on the depression levels in the rats (Tataroğlu et al, 2004).

There is a paucity of studies using the FST paradigm with the bright light manipulation. Molina-Hernandez and Tellez-Alcantra (2000) kept rats on a 14:10 Light/Dark cycle- long photoperiod or 5:19 Light/Dark cycle- short photoperiod for 30 days and compared them with subjects that received a tricyclic (clomipramine or desipramine) antidepressant medication. The ameliorative effect of the long photo period regimen was comparable to the antidepressant efficiency of the tricyclics as measured by the behavioral despair paradigm. The effect of day length on depressive-like behaviors was further addressed by Prendengarst and Nelson (2004) who exposed siberian hamsters to either long photo period (16 hours light/day) or short photoperiod in the beginning of the weaning period (18. day). Two weeks later, behavioral despair was assessed. The results were in favor of the protective effects of long photoperiod on behavioral despair for male hamsters. Interestingly, the short photoperiod hamsters exhibited immobility more rapidly than their long photoperiod counterparts.

A pivotal study by Yılmaz et al (2004) showed the protective effect of light by manipulating 12:12 (light/dark) cycle into a 12:12 (light/light) cycle. The ameliorative role of the light stimulus was evident for the FSTs made immediately after the light exposure or one day later. The rats in both of the conditions were protected from behavioral despair by light stimulus.

Role of parameters of light stimulation in rats has been elucidated by Schulz et al. (2008) in a study that showed that the ameliorative effects of the light stimulation were depended on the time administered (either early or late in the dark period which causes delay and advance respectively), duration and intensity of light stimulation.

The results showed that a 30-minute light pulse delivered 8¼ h after dark onset had an ameliorative effect on the depression levels. This study showed that besides the duration of the photic stimulation, timing of the light pulses has a key role in the antidepressant effects. A recent study from Psychobiology Laboratory at Boğaziçi (manuscript in preparation) showed that 10 minute, 1350 lux bright light stimulation administered at ZT21 (04:00 am) protected the rats from behavioral despair, but the same treatment did not decrease the immobility scores when it was delivered at ZT15 (22:00). ZT21 has a phase advancing quality which is shown to be ameliorative for depression (Terman, 2007).

While the bright light delivered late in the subjective dark period or early in the onset of day has a phase advancing role in circadian rhythm, pulses delivered early in the subjective night delay the phase of the circadian rhythm for the organism (Schibler, 2006). The immediate early genes-PER1 and PER2 expression by the light is correlated with the phase shifting. Accumulation of the PER genes have a role in changing the phase of the molecular clock. The synchronization of the circadian oscillators in other structures and peripheral organs by the SCN is a critical capacity; the lag between the phase adjustments among these organs may have a role in the stress pathology (Schibler, 2006).

The relationship between phase-advance and antidepressant effect were demonstrated by various investigators. Fluoxetine (SSRI) in combination with L-tryptophan was shown to produce phase-advance in the rhythms when applied to the SCN slices (Sprouse et al., 2006).

Wehr et al. (1979) demonstrated that a 6-hour shift for advancing the phase in the depressed patients mimicked the effects of tricyclic antidepressants. The effectiveness of the phase shift hypothesis in these cases raises the expectancy for the

treatment of the depressive patients who are unresponsive to pharmacotherapy. Furthermore, Terman (2007) reported in some of the cases with refractory episodes of depression that bright light delivered early in the morning led to a significant improvement either alone or in combination with antidepressants or alone.

Since light affects the SCN and depression, it is important to elucidate the mechanisms by which light treatment causes amelioration of depression. The SCN is in a position to activate several structures in the limbic system for the antidepressant effect of light. Among these structures, the bed nucleus of the stria terminalis (BNST) is a likely candidate structure for mediatory role of light therapy on depression. The aggravating role of the BNST in the behavioral despair was established (Schulz and Canbeyli, 2004; Pezuk et al., 2006).

The position of the BNST in controlling the visceral and emotional responses and the involvement of the proteins that are involved in the circadian rhythmicity puts this group of nuclei in a unique position in the convergence of the light and stress mediated circadian functions and depression (Amir et al., 2004; Herman and Cullinan, 1997). Uncontrollable stress is an integral part of the depression pathology and BSNT is active during uncontrollable stress (Davis 1998; Walker, Toufexis and Davis, 2003). Therefore, the major cascades that are activated during stressful conditions in the brain and the involvement of the BNST in this brain circuitry will be discussed below. Lastly potential role of the BNST in modulating the effects of photic stimulation on depression pathology will be summarized.

## Stress and Hypothalamic-Pituitary- Adrenal Axis

The term ‘stress’ often connotes damaging and negative qualities that affect the organism. Despite the fact that the transformation of the stressful inputs into the state

of distress is a major concern among investigators of psychopathology research, the moderate levels of stress and the consequent increase in the glucocorticoids in organism is crucial for mobilizing and replenishing the energy for cognitive and body functions (McEwen, 2000).

The wide distribution of receptors for corticosteroid (in human, cortisol) among the limbic structures that are closely related to learning and memory functions highlights the importance of the evaluation of stress signals in the organism and the integration processes in homeostasis condition (Lupien and McEwen, 1997). In line with these arguments, a curvilinear pattern is observed in physiological, cognitive and behavioral outcomes of the cortisol; moderate levels are optimal but the low or high levels are found to be detrimental (Erickson, Drevets and Schulkin, 2003). Motivating effects of the glucocorticoids are also implicated (Plihal et al., 1996).

The role of glucocorticoids in facilitation of the glucose metabolism clearly establishes their pivotal role in many functions such as arousal, reward, attention, learning, memory and sensory-motor coordination. Convergence of these functions under the domain of the stress and its major indicator glucocorticoids in the normal emotional functioning provides clues about the diffuse and complicated network of mechanism in pathological conditions such as depression and anxiety.

In discussing the limbic modulation of the stress and related changes in the brain, one should emphasize the nature of stress, either physical or psychological. This distinction is necessary because there is a stressor-specificity in the modulation of central stress regulation mechanisms (Herman and Cullinan, 1997). The focus of the present study will be on the modulatory effects of psychological stress. Consequences and modulation of the psychological and physiological stress revolve around the HPA axis. Briefly, when a stressor arises, the glucocorticoids regulation

cascade starts with modulation of the neurosecretory neurons of the paraventricular nucleus of the hypothalamus (PVN). These neurons secrete various adrenocorticotrophin (ACTH) secretagogues (CRH, corticotrophin releasing hormone and AVP, arginine vasopressin). These secretagogues follow the hypophysial portal circulation veins to reach to the anterior pituitary. Subsequently, anterior pituitary corticotropes stimulate the ACTH to the systemic circulation. ACTH binds to the adrenal cortex and the glucocorticoids (corticosterone in rat, cortisol in human) are synthesized (Herman et al., 2005; Herman and Cullinan, 1997).

Synthesis and production of the glucocorticoids carry a pivotal role in the survival of the organism towards potential threats, but the balance between the inhibition and excitation mechanisms may actually be more important for the well being of the individual. Hypersecretion of the glucorticoids was found to have detrimental effects on memory, learning capacity and circadian rhythmicity (Austin, Mitchell and Goodwin, 2001). There is also solid evidence that prolonged glucorticoid secretion may lead to neuronal loss and atrophy in certain areas of the brain more specifically the subgenual area of the anterior cingulate, the hippocampus and the prefrontal cortex which may account for the losses in mental faculties in depression such as memory, emotional valencing and executive processes (Drevets et al., 1992).

The focus of the next section will be in the modulatory influences of the bed nucleus of the stria terminalis on the stress circuitry that also is involved in the depression pathology.

# The Bed Nucleus of the Stria Terminalis (BNST)

## Anatomy

The BNST is a ribbon like gray matter that resides in the basal forebrain. Anatomically, the BNST lies ventrally to the lateral septum and dorsally to the preoptic region of the hypothalamus and it surrounds the anterior commissure. The parcellation of subgroups of the BNST shows variations among the researchers, Olmos et al. (2004) made a distinction based on the connectional patterns with the amygdala and other limbic structures such as the nucleus accumbens that eventually creates two major divisions; the central extended amygdala (CEXA) and the medial extended amygdala (MEXA). It is known that morphologically, cytoarchitectonically and chemoarchitectonically, the amygdala, especially the central nucleus of the amygdala has similarities with the BNST; through the stria terminalis and the ansa peduncularis these two structures are functionally connected to each other (Dong et al., 2001). The borders of the extended amygdala change according to different research approaches. Traditionally the BNST is divided into the medial and lateral parts. The medial BNST is also divided into anterior, ventral and posterior parts. The anterior part is placed dorsal to the crossing fibers of the anterior commissure and lies in the dorsal component of the stria terminalis and borders the lateral septum rostromedially. The ventral part is located ventral to the anterior commissure and posterior part reaches to the hypothalamus starting from the uppermost caudal part of the paraseptal area. The lateral BNST is situated ventrally to the posterior aspect of the nucleus accumbens and dorsal to the caudate-putamen (striatum) and surrounds the posterior anterior commissure ventrally (Olmos et al., 2004). A more recent approach for the division of the BNST proposes an anterior/ posterior distinction

(Dong et al., 2001). More than a dozen different nuclei groups are established (for comparison of different approaches of parcellations of the BNST see Dong et al. 2001, p. 197).

A wealth of the afferent and efferent projections of different structures and the presence of a large number of neuropeptides (Gray and Magnuson, 1987), monoamines, steroids and neurotransmitters put the BNST in a distinguished place in stress anatomy.

## Major BNST Projection Areas Involved in Brain Stress Circuitry

Inves tigations using several different labeling procedures have come to the conclusion that the amygdala and the BNST are bidirectionally connected. Particularly, the central and the medial nucleus of the amygdala have projections to the BNST (Weller and Smith, 1982; Cassell, Gray and Kiss, 1986); the central nucleus projections innervate the anterior, whereas the medial amygdala projections innervate the posterior division of the BNST (Dong et al, 2001; Sun, Roberts and Cassell, 1991).

The role of the amygdala in the HPA axis activity and CRH production is a known but still a disputed issue. Particularly, involvement of the BNST in the control of the integration of these projections to the PVN and other hypothalamic areas coupled with a scarcity of direct connections between the hippocampus and the amygdala; highlights the critical role of the BNST as an important relay structure. Kalin et al (2006) presented findings that the destruction of the central nucleus of the amygdala in rhesus monkeys decreases levels of ACTH and cortisol in response to stress compared to the control animals. Additionally, protective effect of electrolytic lesions confined to the central nucleus of the amygdala over behavioral despair was

demonstrated recently in our laboratory (unpublished results). Many of these studies indicate an excitatory influence of the amygdala on the HPA axis and consequent glucocorticoid increases. But the nature of the projections from the amygdala is mostly gamma-aminobutyric acidergic (GABAergic). In line with this, Day et al. (2005) showed that the loud noise and restraint stress caused inhibition in the oval nucleus of the BNST and lateral division of the central nucleus of the amygdala. The extrinsic and intrinsic GABAergic projections between the central nucleus of the amygdala, medial nucleus of the amygdala and the BNST and further projections of the GABA from the BNST to the PVN create a disinhibition state and excites the HPA axis (Herman et al., 2005; Sun and Cassell, 1993). GABAergic influences may be modulated by the interactions between the neuropeptide Y (NPY) and CRH in the BNST (Kash and Winder, 2006). The effects of chronic stress on amygdala and the BNST were demonstrated by Pego et al. (2008) who contended that there was no hypertrophy in the amygdala after stress manipulation. But, morphological analysis of the dendrites revealed hypertrophy in the anteromedial division of the BNST. Hyperanxiety condition may have created more demands for the inhibitory projections that are released from these divisions. The long term effects on the enlargement of these divisions may also show differences due to the lack of adaptive coping ability of the BNST after damaging effects of the prolonged stress (Davis 1998; Davis, 2006; LeDoux et al., 1988; Walker, Toufexis and Davis, 2003).

Hovewer; Fendt, Endres and Apfelbach (2003) found that the temporary inactivation of the BNST by muscimol blocked the freezing response to the trimethylthiazoline (a component of fox feces). In addition to these studies, Shepard, Schulkin and Myers (2006) revealed that elevated levels of the corticosterone in the central nucleus of the amygdala potentiated CRH mRNA in the dorsolateral BNST.

Moreover, in basal conditions exposure to elevated plus maze increased the mRNA in lateral division of the BNST. Tartar, King and Divine (2006) recorded the evoked extracellular field potential responses after electrical stimulation of the BNST. High frequency stimulation (HFS) of the BNST leads to a decrease in the amplitude of PVN field potential responses. Furthermore, HFS application to the dorsolateral BNST created an enduring *N*-methyl- D-aspartate (NMDA) receptor dependent potentiation of the inhibition that caused long term depression in the PVN area possibly by the NR2B receptor subunit of the NMDA.

Evidence that the SCN and the BNST may also be mutually connected in a way through the longer-projecting axons was shown by Allen et al. (1984). In this study the stria terminalis lesions caused marked depletions of the NPY immunoreactivity in the BNST, the SCN and the laterobasal septum. Combined lesions of the preoptic area and the BNST were shown to block the rise in the plasma corticosterone after stimulation of the medial amygdala (Feldman, Conforti and Saphier, 1990).

The hippocampus is known to exert mostly inhibitory influences on the HPA axis, however some excitatory role is also implicated (see Herman et al., 2005). Like other major limbic structures (the amygdala, the prefrontal cortex, the subiculum) that are known to have a vast influence on the HPA axis regulation, the hippocampus has little or no direct projection pathways to the PVN (Choi et al., 2007). The BNST is an important candidate relay area for hippocampal modulation of the HPA axis modulation (Herman and Cullinan, 1997). In line with these, injection of neostigmine in the hippocampus in rats after either having bilateral injections of ibotenic acid or saline were made into the BNST. c-fos expression in the PVN was substantially decreased in the ibotenic-acid injected animals as compared to the vehicle treated animals (Zhu et al., 2001). The inhibitory control of the hippocampus on the HPA

axis is suggested to originate from the neurons in the ventral subiculum-caudotemporal CA1 (Herman et al., 2005). Breakdown of the inhibitory control of the hippocampal modulation under uncontrollable and prolonged stress conditions are implicated during the course of major depression. In addition to the hippocampal modulation, the medial prefrontal cortex control of the PVN may also require the BNST as a relay unit (Spencer, Buller and Day, 2005).

In addition to the complex interaction of the pathways and nature of the influence (inhibitory/excitatory) involving the BNST, this structure also has a dual role during stressful conditions. Anterior or lateral regions of the BNST are implicated in the excitatory influence on the HPA axis, but the medial and posterior portions of the BNST are thought to be inhibitory (Choi et al., 2007. This type of a site dependency in the BNST is compatible with the effects of the connected projection areas on the HPA axis. The central nucleus of the amygdala projections to the anterolateral division of the BNST (Dong et al., 2001) and both the central nucleus and the lateral BNST are implicated in the excitatory impact on the HPA (Herman and Cullinan, 1997) whereas the posterior nuclei receive projections from subiculum (Dong et al., 2001; Herman and Cullinan, 1997).

## BNST and Depression

Involvement of the BNST in the pathology of depression is a recent discovery (Schulz and Canbeyli, 2000; Stout et al., 2000; Pezük et al, 2006; Pezük et al., 2008). There are controversial findings mainly due to the nature of the complexity of the structure and usage of the different behavioral procedures. Stout et al. (2000) found that rats exposed to chronic mild stress had an increase in the CRH peptide concentrations in the BNST. Stressed rats had increased percentages of the threshold

for self-stimulation. The consequences of this increase on the modulation of the other brain areas are not clear; however, there was a trend for decrease in the $CRH_1$ receptor mRNA expression in the cerebellum that did not reached to the significance.

Schulz and Canbeyli (2000) did bilateral electrolytic lesions in rats and assessed behavioral despair using forced swim tests (FSTs). Lesioned animals showed more immobility in the second day of the FST, as compared to sham animals, indicating aggravation of depression and it was shown that the reason for the lack of movement was not due to a general motor impairment. Pezük et al. (2006) confirmed the depressive effect of the BNST lesions and also presented the dissociation between behavioral despair and performance after the BNST lesions. Results showed that there are no detoriation in the navigation abilities of the BNST lesioned animals as compared to the sham-lesioned animals in Morris Water Maze Test. Additionally, freezing response for the uncontrollable aversive tones was found to decrease in BNST lesioned rats (Schulz and Canbeyli, 1999). In line with these findings, Hammack, et al. (2004) also came to the conclusion that the BNST lesions reduced the potentiation of freezing and latency of escaping. These results using fear, anxiety and behavioral despair paradigms, may provide evidence for a critical role of the BNST in emotional states.

## Light-induced Circadian Rhythms

Visual input is conveyed to various areas of the brain for different purposes. Projections from retina reach to the SCN (Moga and Moore, 1997), the ventral lateral geniculate nucleus, the dorsal lateral geniculate nucleus, the lateral posterior/pulvinar nucleus, the pretectum, the superior colliculus and the accessory optic system (van Essevelt et al. 2000; Itaya et al., 1981) Besides these areas, it was shown that there

are two additional direct pathways from the retina; to the BNST and the lateral thalamic nuclei (Itaya et al., 1981). The role of the BNST as a direct projection site is extremely important because it allows the transmission of light-related information into the limbic sites.

Light input is used for the entrainment of the circadian rhythms and thus the SCN is one of the major ultimate targets (Wang et al., 2008). Information from the retina to the SCN by different pathways carries various neurochemical chemical inputs; a direct projection to the SCN by retinohypothalamic tract- glutamatergic, through the intergeniculate nucleus-NPY and the midbrain raphe nucleus-5-HT (van Essevelt et al., 2000; Yannielli and Harrington, 2004). The SCN projects densely to the subparaventricular zone, the paraventricular nucleus of the thalamus (PVT) and the dorsomedial and the ventromedial nuclei of the hypothalamus. It also has smaller projections to the lateral septum and the BNST (van Essevelt et al., 2000). There are reciprocal projections of the SCN with PVT which is also believed to be involved in circadian rhythmicity (Salazar-Juarez, Escobar and Aguilar-Roblero, 2002). Peng and Bentivoglio (2004) showed that synaptic contact from the SCN reached the amygdala through PVT through which Moga, Weis and Moore (1995) showed that the posterior PVT projected strongly to the lateral BNST and the central nucleus of the amygdala. The target areas of these projections in these structures are rich in the CRH neuron density (Li and Kirouac, 2008).

The presence of the multisynaptic pathway between the SCN and adrenal cortex highlights the impact of the light and circadian variations in the stress circuitry. VIP containing SCN projections were labeled in the BNST third order neurons. Interestingly, the PVN and the locus coeruleus have neurons that were second- order labeled (Buijs et al., 1999). Due to the fact that third order neurons

project to second order neurons, the connection between the BNST and the locus coeruleus implicates a possibility for the involvement of the noradrenergic mechanisms in the light mediated antidepressant effects. van Bockstaele, Peoples and Valentino (1999) presented evidence for a monosynaptic pathway between the BNST and the peri-locus coeruleus and the efferent projections from the BNST were originating from medial and lateral parts.

Evidence for the involvement of the BNST in the circadian rhythms as a local clock comes from a study by Amir et al. (2004) study. In the oval nucleus of the BNST (BNST-OV) PER2 protein which is implicated in the photic entrainment (Albrecht et al. 2001) was found to show very strong circadian rhythm and was in synchrony with the SCN. In the arrhythmic SCN-lesioned animals, rhythm of the PER2 expression was not time-dependent and density of the PER2 protein was lower in the BNST-OV. Furthermore, prolonged constant light (LL) was found to blunt the PER2 rhythmicity in the SCN and the BNST. Additionally, in a separate experiment, desynchrony in the PER2 rhythmicity was found to be longer in phase advance than phase delay.

Despite the fact that the BNST is a critical site for the integration of the stressful input and adaptive coping (Schulz and Canbeyli, 2000; Pezük et al, 2006, Henke 1984), implicated in the light mediated circadian rhythmicity (Amir et al., 2004) and antidepressant reactions of neurotransmitters (Duncan et al., 1993), there have been no investigations on the potential role of the BNST as a mediator of the ameliorative effect of light. Therefore, the aim of the present study was to shed light on the anatomical basis for the modulation of the antidepressant effects of the light and circadian rhythms by creating selective lesions confined to the BNST. Wistar rats either had lesions to the BNST or were sham operated. Nine weeks later, a 10

minute 1350 lux light pulse was administered to half of the subjects for each group at ZT21 (04:00). Except for the day of the photic stimulation, rats were maintained on a 12 h light: 12 h dark (lights on at 07:00) lighting cycle. It was hypothesized that, the BNST lesions would cause an aggravation in the behavioral despair in the BNST Lesion-Control (LC) group-that did not receive light treatment. Also in line with the previous findings, sham operated rats receiving light stimulation-Sham-Light group (SL) were expected to be protected against behavioral despair but sham operated subjects not administered light (Sham Control group-LC) were hypothesized to behave similar to the untreated subjects in a typical forced swim test. Lastly, light treatment in the BNST Lesion-Light (LL) group was expected to have no effect on behavioral despair.

# CHAPTER TWO

# METHOD

## Subjects

Thirty-five male Wistar rats bred in the psychobiology laboratory, Boğaziçi University were used in the present study. All of the subjects were experimentally naïve. The range of weights at the start of the experiment was 255 to 370g. Rats were randomly assigned to LC (n: 11), SC (n: 6), LL (n: 11) and SL (n: 7) groups. All animals were group-housed in cages with 4 rats and a 12h:12h dark/light cycle (lights on at 0700 h) was maintained in a temperature controlled environment (22 ± 2 $^{o}$C) with water and food available ad libitum.

## Surgical Procedure

Bilateral lesions of the BNST were performed under anesthesia using 150 mg/kg ketamine and rompun mixture (5mg/2.2mg). Flat skull stereotaxic coordinates relative to bregma were +0.9 anteroposterior (AP), ±1.1 mediolateral (ML) and -6.3 dorsoventral (DV) from dura for every group of rats (Paxinos and Watson, 1997). An anodal current of 1.7mA was applied to both sides of the BNST in durations of 20 to 22 sec. Order of the lesion side was counterbalanced over animals. During sham operations, the electrode was lowered into the same coordinates as for the lesion group without applying any current.

## Light Manipulation

A special insulated light chamber was used for photic stimulation (See Figure 2 below). Nine weeks after surgery, rats were placed in the light chamber in pairs from

the same home cage. Two light chambers were used at the same time. In every light chamber there were two cages and two rats in each cage. Animals were placed in the chamber at 18:50. The light treated subjects were exposed to a 200 watt, 1350 lux bright light for ten minutes at ZT21 (04:00). Control group subjects did not receive any light stimulus during the night in the light chamber. For each group of rats lights were on at 07:00 to create the normal vivarium conditions. Subjects stayed in the chamber until the start of the first forced swimming test at 14:00. After the end of the FST1, rats were placed back in their home cages in the vivarium until the second FST was performed the next day at 14:00.

Fig 2: The light chamber and the other apparatus that were used in the present study

Behavioral Tests

All behavioral tests were performed between 14:00- 16:35 and recorded on a videotape

Forced Swimming Test

Nine weeks after surgery, animals were subjected to two forced swimming tests (FST) separated by 24 hours. FSTs were performed in a Plexiglas cylinder with a height of 45 cm and 30 cm diameter filled with water (25º C) up to the height of 25

cm. Each animal was individually immersed in water for 15 minutes and for 5 minutes in the first and second FST, respectively. After each test, animals were put in a warm place for drying for 30 minutes. For statistical analyses, the first 5 minutes of both tests were used. Immobility defined as the motionless floating behavior of the rat without touching on the walls of the cylinder and was used to assess behavioral despair. Duration of active swimming (consisted of swimming behavior involving continous movement of paws) and frequencies of headshaking, jumps (requires an escape behavior in which half of the body is out of the water) and dives (requires total immersion of the body into the water) which were indicated as struggling behaviors were also recorded.

As an additional method for assessing the different behaviors throughout the FSTs, a new evaluation method by Detke et al. (1995) was used. The counts of immobility, swimming, climbing and diving were coded accordingly to the dominant pattern of behaviors for each 5 sec interval of the 5 minutes swim tests. While increases in the immobility counts indicate an aggravation of behavioral despair, an increase in climbing or swimming counts indicate mobility and amelioration of the behavioral despair.

## Histology

After the end of all behavioral tests, animals were sacrificed with an overdose of ketamine and perfused intracardially by infusion of the 0.9% saline followed by 4% paraformaldehyde into the left ventricle. Brains were removed and fixed with paraformaldehyde solution for several days. After fixation, brains were sliced in the vibrotome with a thickness of 50μm. Sections were put into gelatin-coated slides and stained with cresyl violet.

## Statistics

Results from two different approaches were included in statistical analyses for evaluating behaviors in FSTs. One of them is the conventional method by measuring the immobility and active swimming durations and the number of headshakes for five minutes across FSTs, other type of method is the modified methodology which is consisted of the counting the most dominant pattern of behavior (immobility, swimming and climbing) by 5 sec intervals for five minutes across FSTs. Statistical analyses of two methods and corresponding behavioral parameters depicted in separate headings in the results section.

A 2X2X2 (treatmentxsurgeryxday) repeated measures of analysis of variance (ANOVA) was performed for assessing the effects of treatment (light vs. no light-control) and surgery (lesion vs. sham operated) on durations or frequencies of behavioral parameters of FSTs (day) used in both types of methodology. In addition, a 2X4 (dayxgroup) repeated ANOVAs were used to assess group differences between FST1 and FST2 with respect to each behavior and methodology. Due to the fact that the FST2 is the most crucial part of the behavioral despair paradigm by virtue of being the test phase, one-way ANOVAs were performed to reveal the differences between groups in various behavioral parameters on FST2 in detail.

In addition, immobility ratio criterion (FST2/(FST1+FST2) was used in one-way ANOVA.

The frequencies of diving and jumping behaviors were very small; they were not included in statistical analyses.

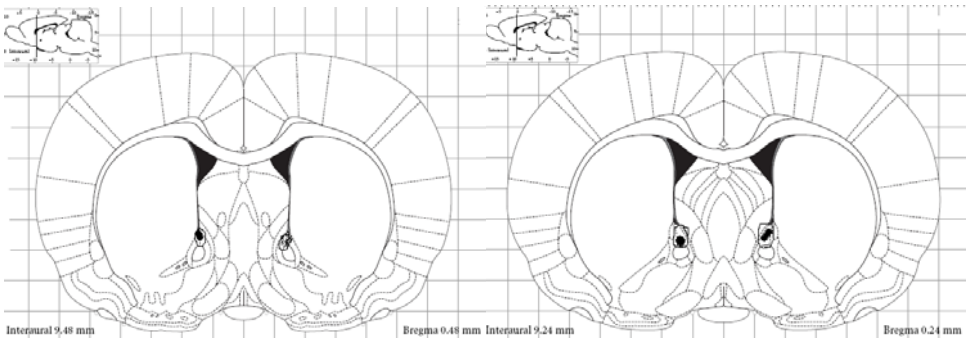
# CHAPTER THREE

# RESULTS

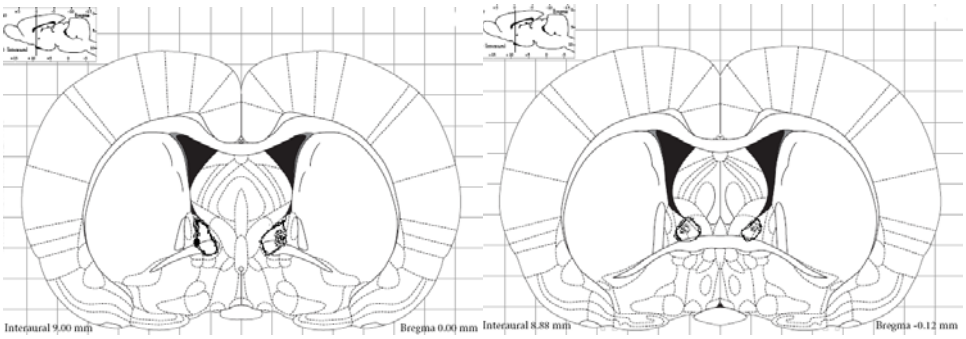
## Histology

Histological analysis with cresyl violet staining revealed that 13 rats out of 22 had symmetrical, bilateral lesions confined to the BNST region with no or very slight damage to the adjacent tissue (Fig. 3). Of the remaining 9 lesioned rats, 7 were found to have unilateral lesions and one was mislesioned. Another animal was lesioned more anterior and ventral with respect to the BNST lesion groups. Therefore, these animals were excluded from the statistical analyses.

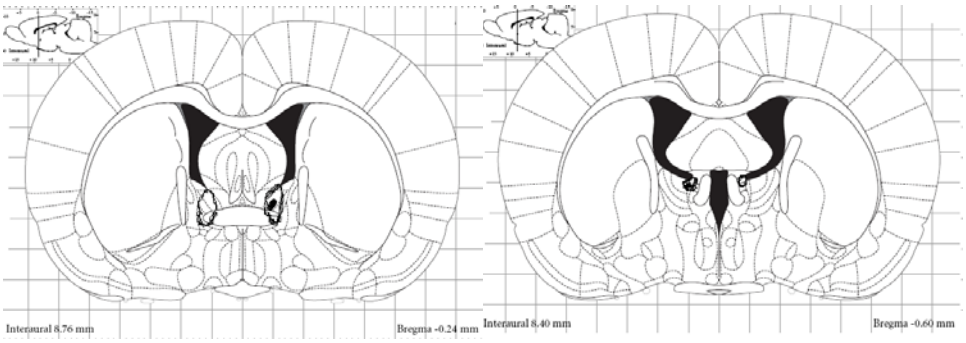
The distribution of lesioned rats into two lesion groups was 7 in the LC group and 6 on the LL group. In these animals, lesions were discrete with very slight differences between two sides of the BNST and with little or no invasion to the stria terminalis and the anterior commissure. The lesions were confined to the medial aspect of the BNST in its rostrocaudal extension according to the nomenclature by Olmos et al. (2004). Drawings of the lesion sections for each animal are presented in the Appendix.




Bregma 0.48 mm Bregma 0.24 mm




Bregma 0.00 mm Bregma -0.12 mm




Bregma -0.24 mm Bregma -0.60 mm

Fig. 3: Sections of the rat brain showing the smallest (in black) and the largest (depicted in outline) lesions of the BNST. Brains were adapted from Paxinos and Watson (1997).

## Forced Swim Test

### Conventional Methodology

Means (Mean ± SEM) of immobility, active swimming durations and number of headshakes in the two forced swim tests for the four groups are presented in Table 1.

Table 1
Total Durations of (Mean + SEM) Immobility and Active Swimming and Frequencies of Headshakes in Forced Swim Tests (FSTs) Performed 24 h Apart for Lesion- Light, (LL, n=6), Lesion-Control (LC, n=7), Sham-Light (SL, n=7) and Sham-Control (SC, n=6) Groups.

| | Immobility | | Active Swimming | | Head Shakes | |
|---|---|---|---|---|---|---|
| | FST1 | FST2 | FST1 | FST2 | FST1 | FST2 |
| LL | 50.33± 2.08 | 46.67 ± 5.96 | 80 ± 7.15 | 79.67 ± 12.30 | 46.17 ±3.99 | 55.50 ± 4.36 |
| LC | 63.14 ± 10.92 | 90.57± 11.60 | 84.14 ± 7.89 | 67.29± 10.21 | 42.29 ± 4.20 | 45.14 ±4.28 |
| SL | 68 ±5.18 | 49.86 ±6.43 | 90.71 ±9.75 | 102.14 ±18.31 | 35.43 ± 4.52 | 44.86 ±2.46 |
| SC | 61.83 ± 4.32 | 65.50 ± 5.85 | 93 ± 4.12 | 103.17 ±16.57 | 33.50 ± 4.75 | 38.33 ±2.85 |

## Immobility

Figure 4 presents the immobility durations of four groups in FSTs.

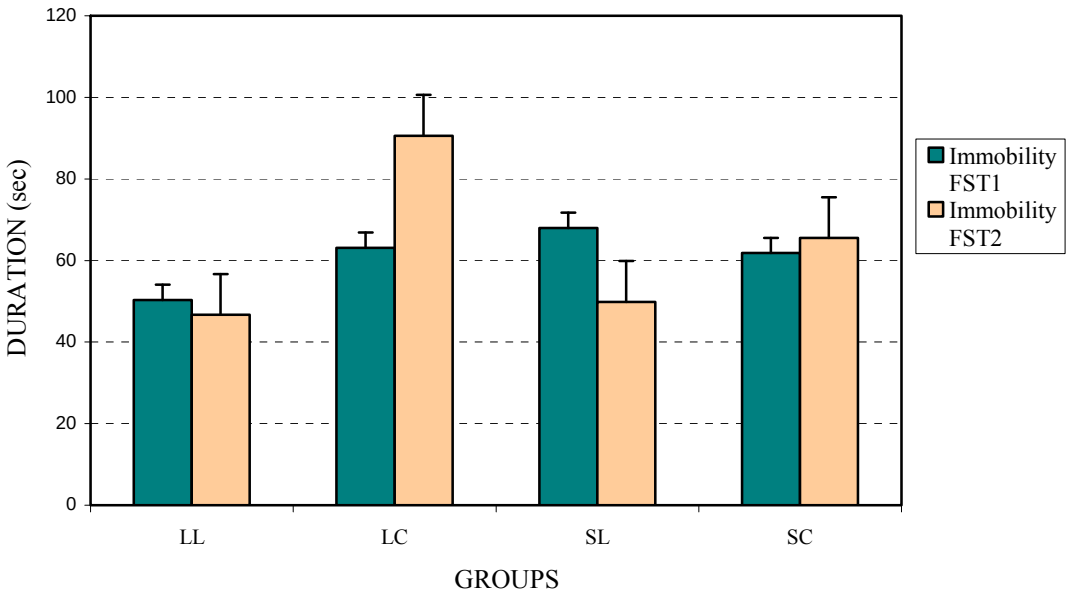



Fig. 4: Durations of the immobility across two forced swim test for LL (n=6), LC (n=7), SL (n=7) and SC (n=6) groups in seconds.

A 2X2X2 (treatment X surgery X day) ANOVA with repeated measures showed that there were no difference between FST1 and FST2 with respect to immobility durations [F (1, 22) = 0,601, p>0.05). There were significant interactions between day X treatment [F (1, 22) = 10,198, p<0.004] and day X surgery [F (1, 22) = 19,522, p<0.000]. But, treatment X surgery X day interaction did not reach to significance [F (1, 22) =0,601, p>0.05)].

Between subjects evaluation revealed a main effect of treatment [F (1, 22) = 5,659, p<0.039] but no main effect for surgery [F (1, 22) = 0,601, p>0.05] and interaction between surgery and treatment was not significant [F (1, 22) = 2,882, p>0.05]. Thus, light treatment had the same effect in lesioned and sham-operated rats.

To elucidate the differences among groups a 2X4 (day X groups) ANOVA with repeated measures was performed. There was a significant interaction between dayxgroup with respect to immobility durations [F (3, 22) = 10,894, p<0.000] and there was a significant difference between groups in immobility durations [F (3, 22) = 2.896, p<0.05]. Post-–hoc test LSD revealed that the actual difference was between LC and LL groups (p<0.009).

One-way ANOVA indicated no difference between groups in the FST1 [F (3, 25) = 1,141, p>0.05], but for the FST2, between subjects effect was significant, [F (3, 25) = 6,307 (p<0.003)]. LSD post-hoc test revealed a significant difference for immobility durations between LL-LC (p<0.001), LC-SL (p<0.001), LC-SC (p<0.041) groups. Difference between SL-SC group reached to significance by one-tailed Student's T-test [T (11) = -1,774, p<0.05].

Immobility ratio criterion for assessing the distinction between groups was used in one way ANOVA. Results indicated a significant difference between groups for the immobility ratios [F (3, 25) = 8,004 (p<0.001)]. Post hoc tests revealed a significant difference among the LL-LC (p<0.005), LC-SL (p<0.000), LC-SC (p<0.045) and SC-SL (p< 0.021) groups, confirming the difference between the groups in FSTs.

Active Swimming

Durations of active swimming for four groups in FSTs were provided in Figure 5.

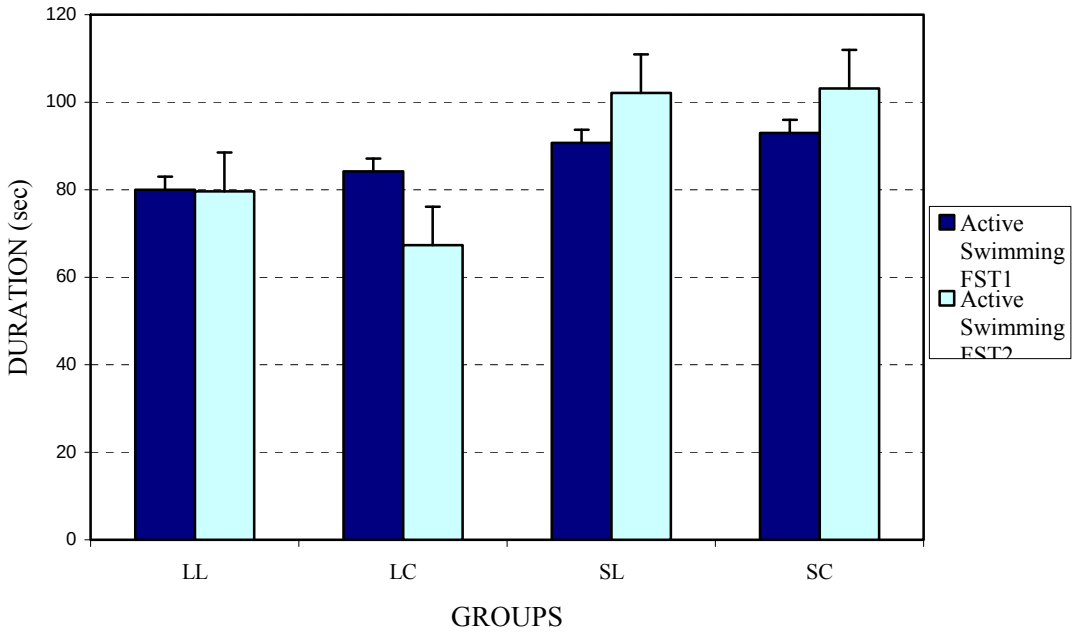



Fig.5: Active swimming durations in two forced swimming tests for LL, (n=6), LC (n=7), SL (n=7) and SC (n=6) groups in seconds.

A 2X2X2 (treatment X surgery X day) ANOVA with repeated measures showed no significant difference between FST1 and FST2 with respect to active swimming durations [F (1, 22) = 0,030, p>0.05]. There were also no interactions between factors, day X surgery [F (1, 22) = 2,359, p>0.05]; day X treatment [F (1, 22) = 0,496, p>0.05]; day X surgery X treatment [F (1, 22) = 0,365, p>0.05]. Main effect of surgery approached significance [F (1, 22) = 3,784, p=0.065], but main effect of treatment [F (1, 22) = 0,015, p>0.05] or treatment X surgery interaction [F (1, 22) = 0,083, p>0.05] were not significant. Also there were no significant group differences [F (3, 22) = 1,314, p>0.05] by 2X4 (day X groups) ANOVA with repeated measures or one-way ANOVA in FST1 [F (3, 25) = 0,563, p>0.05] and FST2 [F (3, 25) = 1,457, p>0.05].

Headshakes

Figure 6 shows the number of headshakes in FSTs for four groups.

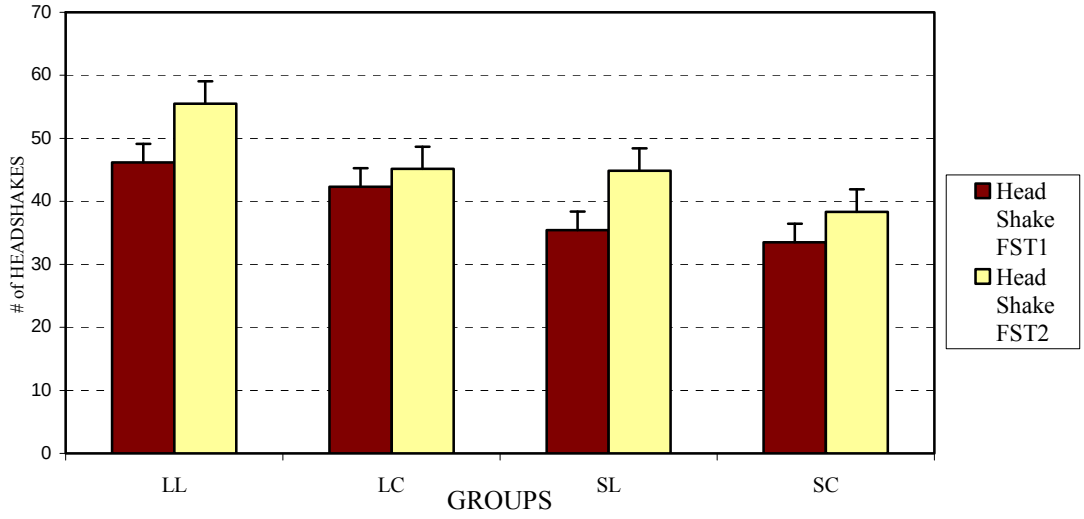



Fig.6: Number of headshakes performed during two forced swimming tests for LL (n=6), LC (n=7), SL (n=7) and SC (n=6) groups.

A 2X2X2 (treatment X surgery X day) ANOVA with repeated measures yielded significant difference between FST1 and FST2 in frequency of headshakes [F (1, 22) = 8,077, p<0.009], but no significant interactions between factors, day X surgery [F (1, 22) = 0,050, p>0.05]; day X treatment [F (1, 22) = 1,415, p>0.05]; day X surgery X treatment [F (1, 22) = 0,041, p>0.05]. A main effect of surgery between subjects was shown [F (1, 22) = 7,969, p<0.010], however, there was neither a main effect of treatment [F (1, 22) = 0,097, p>0.05] nor a significant interaction between treatment X surgery [F (1, 22) = 0,195, p>0.05].

A 2X4 (day X group) ANOVA with repeated measures indicated a main effect of group with respect to frequency of headshakes [F (3, 22) = 3,491, p<0.03]. Post hoc comparisons using LSD test indicated that difference was between LL-SL (p<0.031) and LL-SC (p<0.005).

One-way ANOVA indicated no difference between groups in the FST1 [F (3, 25) = 1,739, p>0.05], but for the FST2, between subjects effect was significant, [F

(3, 25) = 3,622, p<0.029] in terms of headshake frequencies. Besides, LSD post-hoc test revealed a significant difference for immobility durations between LL-LC (p<0.05), LL-SL (p<0.048), LC-SC (p<0.004) groups. LL group did more headshakes than any other groups, therefore presented more struggling in FST2.

## Modified Methodology

Frequencies (mean ±SEM) of immobility, swimming and climbing counts for different groups in two consecutive days of FSTs are given in Table 2.

Table 2
Frequencies (Mean + SEM) of Climbing, Swimming and Immobility Counts Assessed by the New Methodology in Forced Swim Tests (FSTs) for LL, (n=6), LC (n=7), SL (n=7) and SC (n=6) Groups.

| | Climbing | | Swimming | | Immobility | |
|---|---|---|---|---|---|---|
| | FST1 | FST2 | FST1 | FST2 | FST1 | FST2 |
| LL | 2.17 ± 0.75 | 1.00 ± 0.37 | 17.00 ± 2.12 | 19.67 ± 1.47 | 40.83 ± 2.07 | 39.33 ±1.31 |
| LC | 1.00 ± 0.65 | 2.29 ± 1.21 | 17.14 ± 2.16 | 10.86 ± 2.00 | 41.14 ± 1.91 | 46.57 ± 2.35 |
| SL | 7.29 ± 1.46 | 7.71 ± 2.85 | 18.57 ± 1.75 | 16.86 ± 2.69 | 34.86 ± 2.12 | 36.43 ±2.70 |
| SC | 6.50 ± 2.36 | 2.17 ± 1.01 | 21.17± 2.18 | 18.33 ±2.27 | 32.33 ± 2.50 | 39.50 ± 2.72 |

### Immobility

Figure 7 presents the frequencies of immobility counts in FSTs for four groups

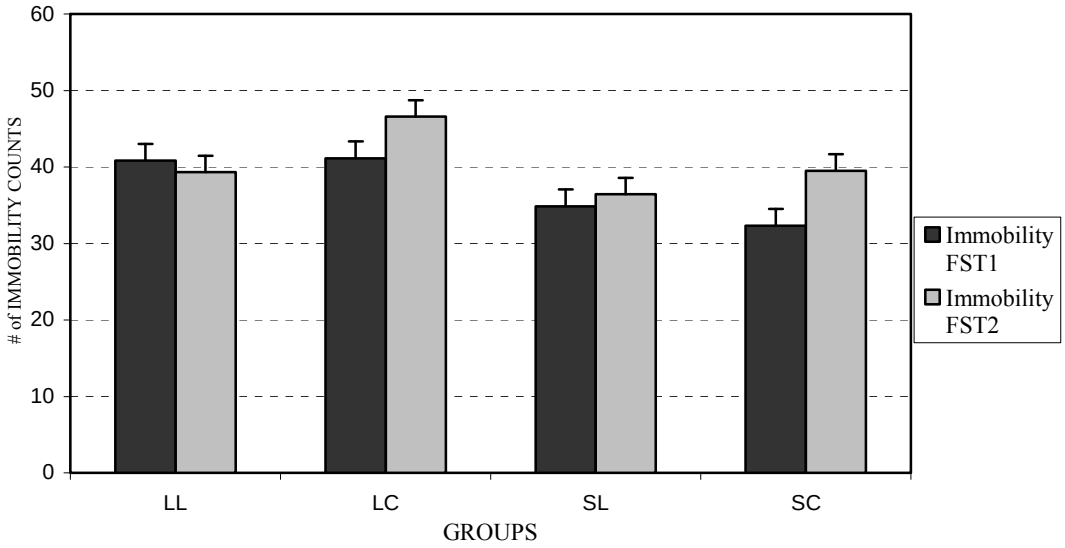



Fig. 7: Number of immobility counts for LL (n=6), LC (n=7), SL (n=7) and SC (n=6) groups in forced swim tests.

A 2X2X2 (treatment X surgery X day) ANOVA with repeated measures showed that difference between FST1 and FST2 with respect to immobility counts were significant, [F (1, 22) = 7,687, p<0.011]. There was a significant day X treatment interaction [F (1, 22) = 7,515, p<0.012] but no significant interaction between day X surgery [F (1, 22) = 1,108, p>0.05] and between treatment X surgery X day [F (1, 22) =0,085, p>0.05].

Between subjects evaluation revealed a main effect of surgery [F (1, 22) = 9,929, p<0.001], but there was no main effect of treatment [F (1, 22) = 0,314, p>0.05] and interaction between surgery and treatment was not significant [F (1, 22) = 0, 794, p>0.05].

To illuminate the differences among groups, a 2X4 (day X groups) ANOVA with repeated measures was conducted. There was no significant interaction between day X group with respect to immobility counts, although this fell just short of significance level was approaching to significance [F (3, 22) = 2,771, p=0.06]. In addition, there was a main effect of group in immobility counts [F (3, 22) = 4,117,

p<0.018]. Post-–hoc test LSD revealed that actual difference was between LL-SC (p<0.009) and LC-SL (0.006).

One-way ANOVA indicated a significant difference between groups in FST2, [F (3, 25) = 3,513 p<0.03]. Post hoc comparisons with LSD revealed a significant difference for immobility counts between LL-LC (p<0.04), LC-SL (p<0.005), LC-SC (p<0.048) groups. There was no difference between SL-SC groups.

Swimming

Figure 8 presents the frequencies of swimming counts in FSTs for four groups

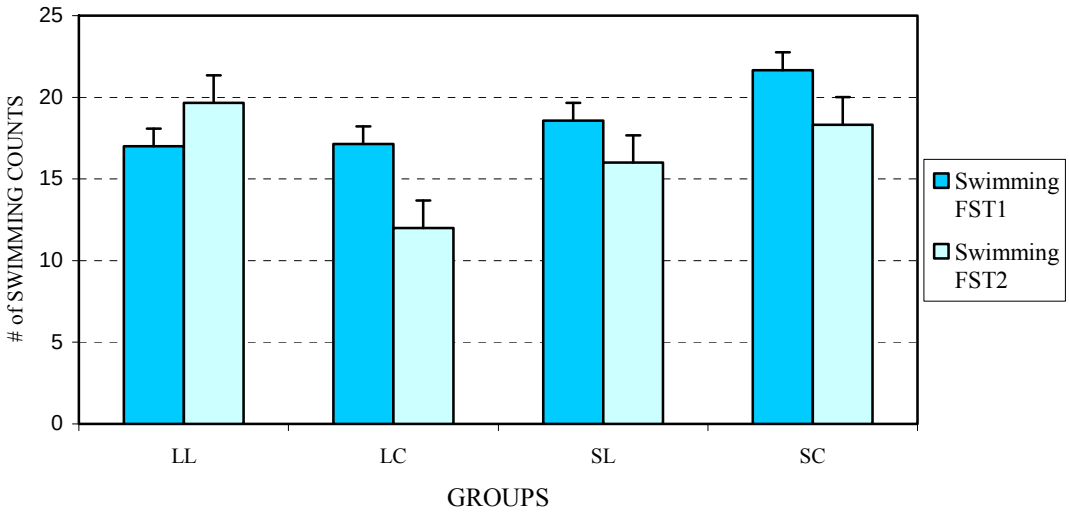



Fig 8: Number of swimming counts for LL (n=6), LC (n=7), SL (n=7) and SC (n=6) groups in forced swim tests.

A 2X2X2 (treatment X surgery X day) ANOVA with repeated measures indicated no significant difference between FST1 and FST2 with respect to swimming counts, but the difference approached significance [F (1, 22) = 3,247, p= 0.085]. In addition, interaction between day X treatment did not reach to significance [F (1, 22) = 3,985, p=0.079]. There were no further significant interactions between day X surgery [F (1, 22) = 0,127, p>0.05] and between treatment X surgery X day [F (1, 22) =3,011, p=0.097]. In between subjects effects, interaction between surgery X treatment was marginally significant [F (1, 22) = 4,236, p=0.052] without any main

effects of surgery [F (1, 22) = 2,027, p>0.05] or treatment [F (1, 22) = 0,320, p>0.05].

Also, a 2X4 repeated measures ANOVA did not yield any significant differences between groups in swimming counts [F (3, 22) = 2,241, p>0.05]

Contrary to the conventional methodology reported above, the modified method revealed a significant between subjects effect in one-way ANOVA for FST2 [F (3, 25) = 3,556, p<0.031]. Post hoc comparison with LSD showed that LL-LC (p<0.007) and LC-SC (p<0.019) groups were different from each other in swimming.

Climbing

Figure 9 shows climbing frequencies for each group in FST1 and FST2.

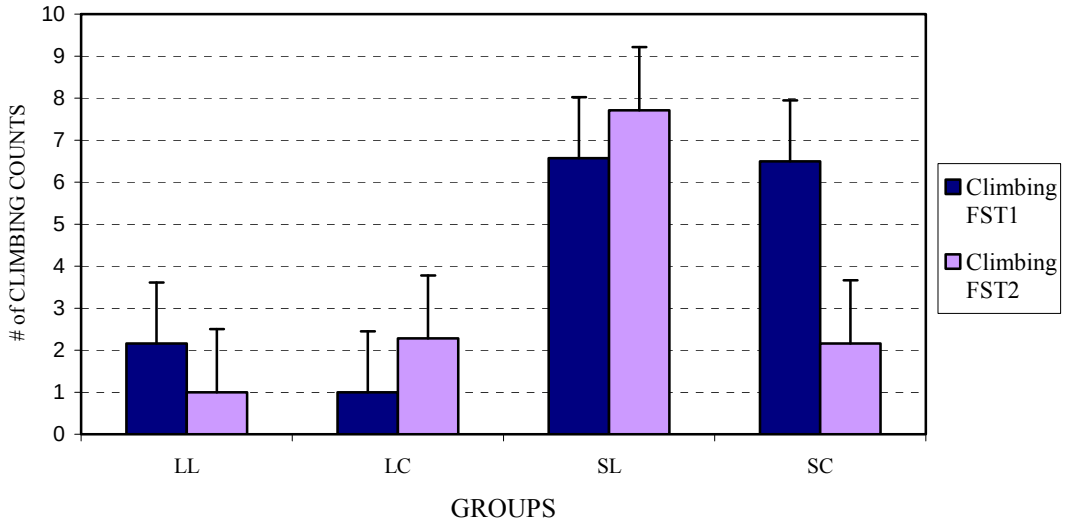



Fig. 9: Number of climbing counts for LL (n=6), LC (n=7), SL (n=7) and SC (n=6) groups in forced swim tests.

A 2X2X2 (treatment X surgery X day) ANOVA with repeated measures indicated no difference between FST1 and FST2 with respect to climbing counts [F (1, 22) = 1,231, p>0.05]. Results yielded a significant interaction between treatment X surgery X day [F (1, 22) = 8,200, p<0.009]. Interactions between day X treatment [F (1, 22) = 1,193, p>0.05] and day X surgery [F (1, 22) = 1,429, p>0.05] were not significant. In addition, main effect of surgery was demonstrated [F (1, 22) = 7,834,

p< 0.010]. Main effect of treatment [F (1, 22) = 0,870, p>0.05] and treatment X surgery interaction [F (1, 22) = 0,947, p>0.05] did not reach to significance.

To delve into group differences, a 2X4 repeated measures ANOVA was performed. Dayxgroup interaction was significant [F (3, 22) = 3,545, p<0.031]. Furthermore groups were significantly different from each other [F (3, 22) = 3,369, p<0.037]. LSD test revealed that difference was for LL-SL (p<0.014) and LC-SL (p<0.012) groups.

One way ANOVA for FST2 yielded a significant between subjects difference for climbing [F (3, 25) = 3,060, p<0.049]. Post hoc comparison using LSD tests, revealed that LL-SL (p<0.013), SL-SC (p<0.036) and SL-LC (p<0.033) were significantly different from each other.

Lastly, to compare the two methodologies in assessing the difference between FST1 and FST2 for immobility visually, the means for immobility durations and counts with the respective significance levels is provided in the Table 3.

Table 3: Comparison of the Two Methodologies in Evaluating the Differences Between First Day of the Forced Swim Test and Second Day of the Forced Swim Test with Respect to Immobility Behavior. Significance Values were Assessed by Two-tailed Paired Samples t-tests.

| | Immobility Duration | | Significance | Immobility Count | | Significance |
|---|---|---|---|---|---|---|
| | FST1 | FST2 | FST1-FST2 | FST1 | FST2 | FST1-FST2 |
| LL | 50.33 | 46.67 | 0.6 | 40.83 | 39.33 | 0.6 |
| LC | 63.14 | 90.57 | 0.000** | 41.14 | 46.57 | 0.02* |
| SL | 68 | 49.86 | 0.04* | 34.86 | 36.43 | 0.36 |
| SC | 61.83 | 65.50 | 0.6 | 32.33 | 39.50 | 0.04* |

# CHAPTER FOUR

# DISCUSSION

The aim of the present study was to delineate the anatomical basis for the antidepressant effect of light by destruction of a specific structure, the BNST, which has a critical role in stress circuitry (Herman et al., 2005). Light-mediated antidepressant actions induced by 200 watt, 1350lux 10-min photic stimulus delivered at a very specific hour of the subjective night (ZT-21, 04:00) after nine weeks from surgery (lesion of the medial BNST or sham-operation) was the focus of the present thesis. Another contribution of this study was to provide a comparison between two different methodologies used in assessing behavioral despair.

The findings of the present study confirmed an aggravation of behavioral despair in the BNST-lesioned (LC) group as measured by two consecutive forced swimming tests. This finding constitutes a replication of the previous studies in the Psychobiology Laboratory which established an augmentation of the immobility durations from first day of the swim test to the second day in rats with the BNST lesions which is confined mostly to the medial division of the BNST (Schulz and Canbeyli, 2000; Pezük et al., 2006). Statistical analysis of the results from modified methodology was parallel to the conventional assessments; immobility durations and immobility counts were shown to be significantly increased from FST1 to FST2 in LC group. By performing the modified methodology, the significant increase from FST1 to FST2 in SC group with respect to immobility counts was evident. But there was no such augmentation of the immobility behavior observed in SC by conventional method. Also both methodologies indicated a significant difference

between LC-SC groups with respect to immobility in FST2, LC group had the highest immobility durations among groups. For swimming behavior, only the modified methodology yielded a significant difference between LC-SC groups; frequencies of swimming counts were greater in SC group. These findings are compatible with the role of the medial BNST as an inhibitory relay structure for HPA axis and thereby acting as a coping mechanism in depression (Choi et al., 2007; Pezük et al, 2006, Pezük et al, 2008). In contrast, lateral parts were considered to have an excitatory influence on the HPA axis when organism is triggered with stress (Herman et al., 2005).

The critical inquiry of the present study was the role of the BNST lesions in antidepressant effect of bright light therapy. To start with, results confirmed the ameliorative effect of short light pulses administered to sham and lesioned (SL and LL) groups as compared to those in the sham and lesion control groups, namely SC and the LC groups. The efficacy of the 10 min. light pulses delivered late at the subjective night (ZT21) as a protection against behavioral despair was demonstrated recently in our laboratory (manuscript in preparation). Photic stimulation had a robust antidepressant effect by creating a phase advance for rats in LL and SL groups. The conventional methodology based on duration of immobility was a better indicator of the antidepressant effect of light in SL group; it yielded a significant decrease from the first day to the second day of swim test. For LL group there was no difference among FSTs. In the modified methodology, both LL and SL groups did not show an increase or decrease of immobility between days.

Group differences assessed by both types of methods indicated a significant difference between LL and LC groups for FST2; light had an ameliorative effect for LL group in terms of decreased levels of immobility and increased number of

headshakes and swimming counts. Despite the fact that there was no difference between SL and SC groups in one-way ANOVA, parallel to the hypothesis, a one-tailed t-test yielded a significant difference between SL-SC groups with the conventional methodology. To strengthen the presence of the difference between SC and SL groups, comparison of the immobility ratios (FST2/(FST1+FST2) between groups indicated a significant difference between SC and SL groups; the rats in the SL group were found to be protected from the aggravagating impact of the BNST lesion by the 200 watt, 1350 lux, 10 minutes light exposure.

Upon evaluation of the climbing and swimming frequencies with modified methodology, a possible dissociation between the effects of factors; treatment and surgery was observed. In the LC group, frequency of swimming counts decreased as it was expected and in the LL condition, light potentiated swimming. But in the climbing parameter, the picture was different. SL group had the highest climbing frequency in FST2, but the climbing counts of LL group as compared to the LC group were not differed from each other, light did not cause amelioration in the climbing behavior. Potential explanations for these findings could be as follows. First, serotonergic modulation of the antidepressant effect of light may be preserved in the lesioned rats, which is in line with the role of the lateral and ventral part of the BNST in the serotonergic modulation and these portions were largely intact in the present experiment. The involvement of the serotonergic systems in antidepressant action is bolstered by a study presented by Greenwood et al. (2005) emphasizing the crucial role of the BNST in prevention of learned helplessness after 6 weeks of wheel running; especially, stress-induced c-fos expression was attenuated in the lateral ventral region of the BNST and in the dorsal DRN in the rats that were engaged in physical activity. Second, noradrenergic modulation of the antidepressant effect of

light may have been dampened in the lesioned rats such that the light pulse could not compensate for the alleviation of this behavior as a part of the antidepressant response. This kind of dissociation was also shown by the Cryan and Lucki, 2000 (also, Page et al., 1999) while frequencies of immobility counts decreases after serotonergic or noradrenergic antidepressant mechanisms, swimming increased in the presence of the serotonergic antidepressants and climbing frequencies were increased after catecholaminergic modulation of the antidepressant.

The present study revealed that lesions confined to the medial part of the BNST do not prevent the antidepressant action of short light pulses at ZT21. Therefore, medial aspect of the BNST may not be a critical site for the modulation of the stress and light interaction due to the fact that the protective effect of the light was still evident in LL animals. These results are to be expected in light of the compartmentalized action mechanism of the BNST towards stress such that medial parts of the BNST are implicated in inhibitory influences to the HPA-axis, but the lateral parts are indicated in excitatory influences (Herman et al., 2005). A similar kind of compartmentalized mediation in antidepressant actions may be observed in the BNST.

Despite involvement of the BNST in antidepressant mechanisms, the results of the present study did not support for a mediatory role of the medial division of the BNST in the antidepressant actions of the light. None of the studies described above used behavioral despair paradigm in measuring the stress levels and also none of them used the light to achieve an antidepressant response. Therefore, differences between the behavioral paradigms in measuring the stress prevent to reach to conclusion about the involvement of the BNST in amelioration of behavioral despair. Besides, the BNST may have distinct responses to various antidepressant agents.

Involvement of the SCN in light mediated circadian functioning (Tataroğlu et al, 2004; Turek, 2001) is known, but the role of the BNST in circadian rhythms and corresponding changes in the HPA axis signaling and CRH modulation after photic stimulation have not been elucidated yet. Amir et al. (2004) demonstrated a SCN-coupled PERIOD2 protein expression on the Oval Nucleus of the BNST (situated in the lateral portion of the BNST) and they further showed entrainment after a phase-advance was found to have a differential timing for the BNST, providing further evidence for the possible involvement of the BNST in the antidepressant effect of the phase-advance. Furthermore, Yamazaki et al. (1998) recorded multiple unit neural activity from inside and outside of the SCN. A unique phase relationship between the BNST and the SCN was demonstrated; there was a strong coupling between the BNST and the SCN and the circadian and ultradian rhythms were always in-phase for these two structures. This kind of an effect was not observed for other brain structures. The authors suggested the BNST as a major output projection area from the SCN to the locomotor centers. Despite strong association between the SCN and the BNST, coupling properties of these structures may subserve the rhythmic modulation of the other functions of the BNST such as the modulation of sexual behaviors by light (Raitiere et al., 1995).

Circadian rhythms provided by the SCN affect many physiological functions such as temperature, locomotor activity, adrenal corticosterone production and pineal melatonin secretion (van Essevelt et al., 2000). Tracing studies showed that the SCN involved in the HPA-axis circuitry and glucocorticoid secretion in multiple steps. In addition of a direct connection to the PVN neurons and CRH production, it is also connected to the dorsomedial nucleus of the hypothalamus. Buijs et al. (1999) demonstrated that, the PVN/DMH (the dorsomedial hypothalamus) works as an

integration area for the SCN information to be transmitted to melatonin and corticosterone regulation and the regulation of corticosterone secretion was dependent on the timing of the light stimulus. While light given earlier in the subjective night (ZT 14, 15 minutes) has an inhibitory influence on the corticosterone secretion, photic stimulus presented late at the subjective night (ZT20, 15 minutes) does not have such an effect. Retrograde tracing studies of the SCN projection sites also revealed an innervation of the peri SCN region. The SCN is also modulated by feedback loops (van Essevelt et al., 2000). When the basal corticosterone levels are low, the SCN inhibits the corticosterone responsiveness, but when the corticosterone levels are high, it may by pass the intermediary steps and regulate through the adrenal gland response (Sage, Maurel and Bosler, 2001).

In line within evidence, consequences of the medial BNST lesions may have created such a regulation involving the adrenal gland by eliminating the intermediary steps. Stout et al. (2000) had shown that after 19 days of chronic mild stress, CRH concentrations in the BNST was increased significantly as compared to the controls. Increased levels of CRH concentrations provide clues for the insufficient inhibitory control mechanisms. The medial division of the BNST is one of the major targets for the inhibitory control of the HPA-axis and consequent glucocorticoid feedback mechanisms. Assuming a breakdown of coping ability of the BNST during stress and thus lack of inhibitory control over the HPA-axis, the BNST lesioned status may be thought as mimicking the actual depressive state. The resultant increase in the CRH concentrations may trigger the adrenal gland response after the photic phase shift. The intermediary steps between the photic shift and the antidepressant response require further investigation. The efficacy of the bright light therapy resulting in a phase advance in refractory or treatment resistant depression (Terman, 2007) may be

a result of these complex mechanisms that are modulated by prolonged stress and abnormal CRH levels. BNST-lesion mediated behavioral despair in the rat may resemble the refractory and non-responding chronic depression states and the consequent alleviation of the behavioral despair by light may also provide an explanation for the efficacy of the bright light therapy especially in refractory depression types.

Output pathways of the SCN that are suggested to be involved in the HPA – axis and stress mediation other than the BNST may also be involved in antidepressant effect of light stimulation. Candidate areas for this type of a modulation are the PVN, the lateral septum and the PVT. In line with this explanation, Salazar-Juarez (2002) showed that lesions of the anterior part of the PVT abolish the phase advance in rats measured by drinking behavior.

Results of the present study confirms the antidepressant effect of the light and provides solid evidence for the efficacy of bright light therapy in depression by mimicking the depressive state in the human by performing BNST lesions which is implicated to be disturbed during chronic uncontrollable stress conditions. Therefore, ameliorative effect of the short light pulses in the LL group brings out the possible involvement of a large scale complex but integrated action mechanism for the mediation of the antidepressant role of the light.

# CHAPTER FIVE

# CONCLUSION

BNST is an integral part of the stress related circuitry and also highly implicated in modulations of circadian rhythms. The results from the present thesis did not support a role of the medial division of the BNST in the light mediated antidepressant effect. The afferent and efferent projections around the BNST increase the complication of the compartmentalized reactions of the BNST towards stress. Short light pulses delivered at ZT21 produced a robust antidepressant effect in both BNST-lesioned and sham operated animals. This study is the first report for the role of the BNST in the mediation of ameliorative effects of the light. The efficacy of the bright light therapy in non-seasonal depression was established.

APPENDIX

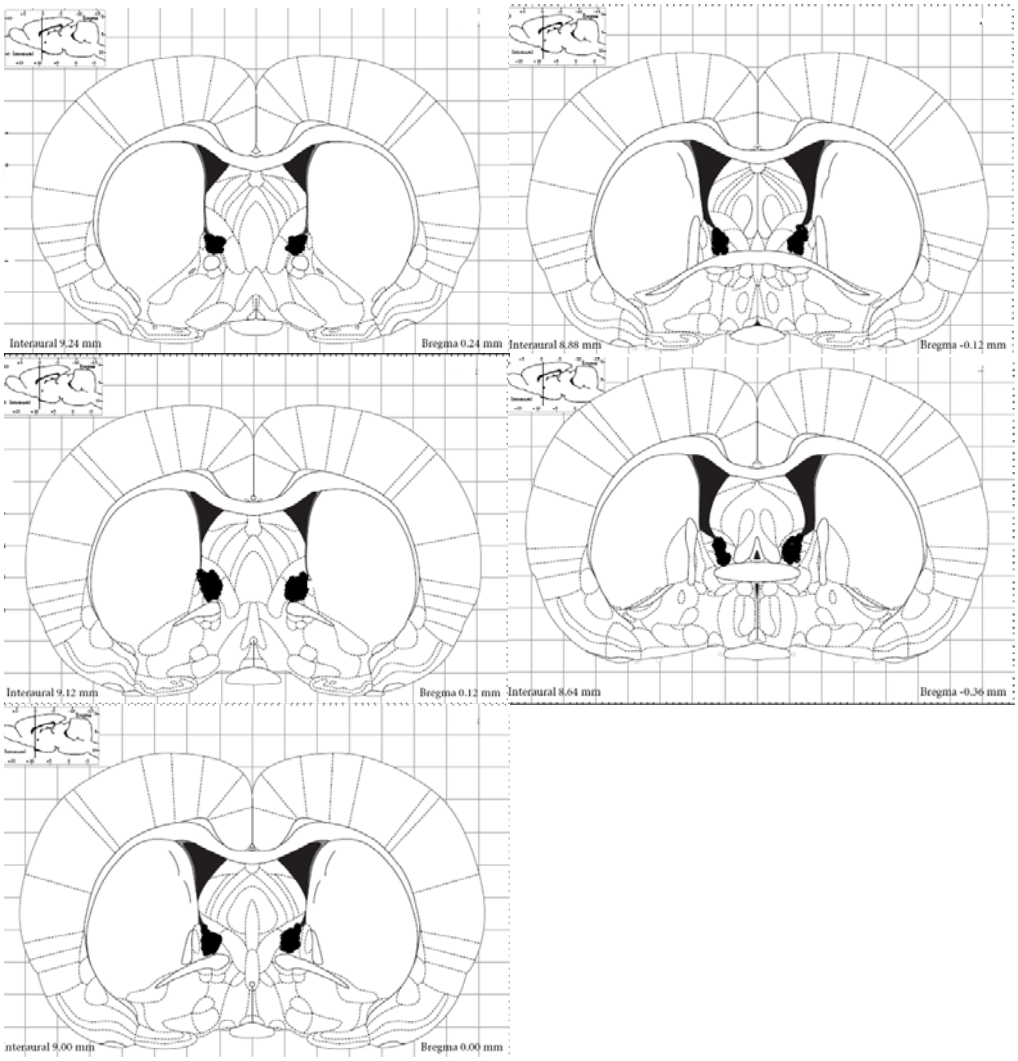



Figure 1: BNST lesion for the #20. Brain Maps are adapted from Paxinos and Watson, 1997

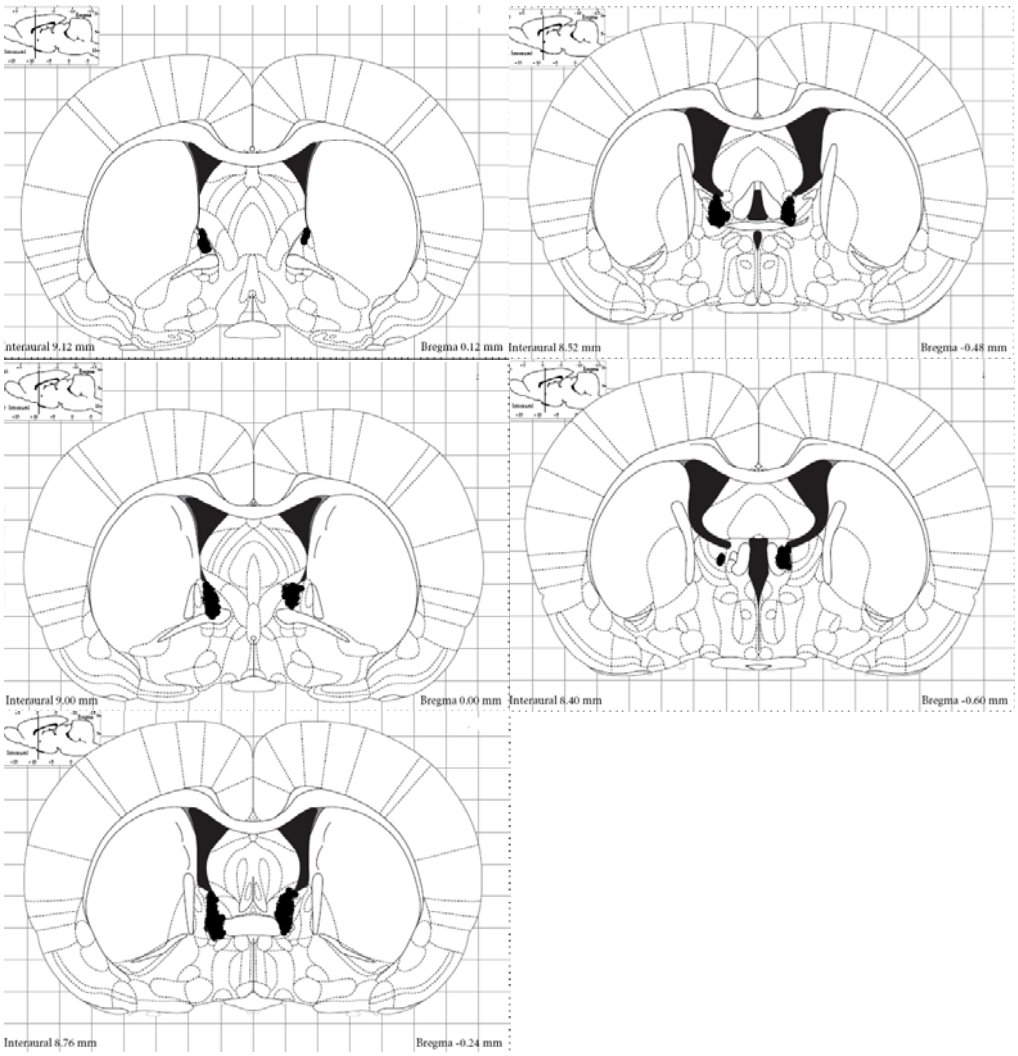



Figure 2: BNST lesion for the #30. Brain Maps are adapted from Paxinos and Watson, 1997

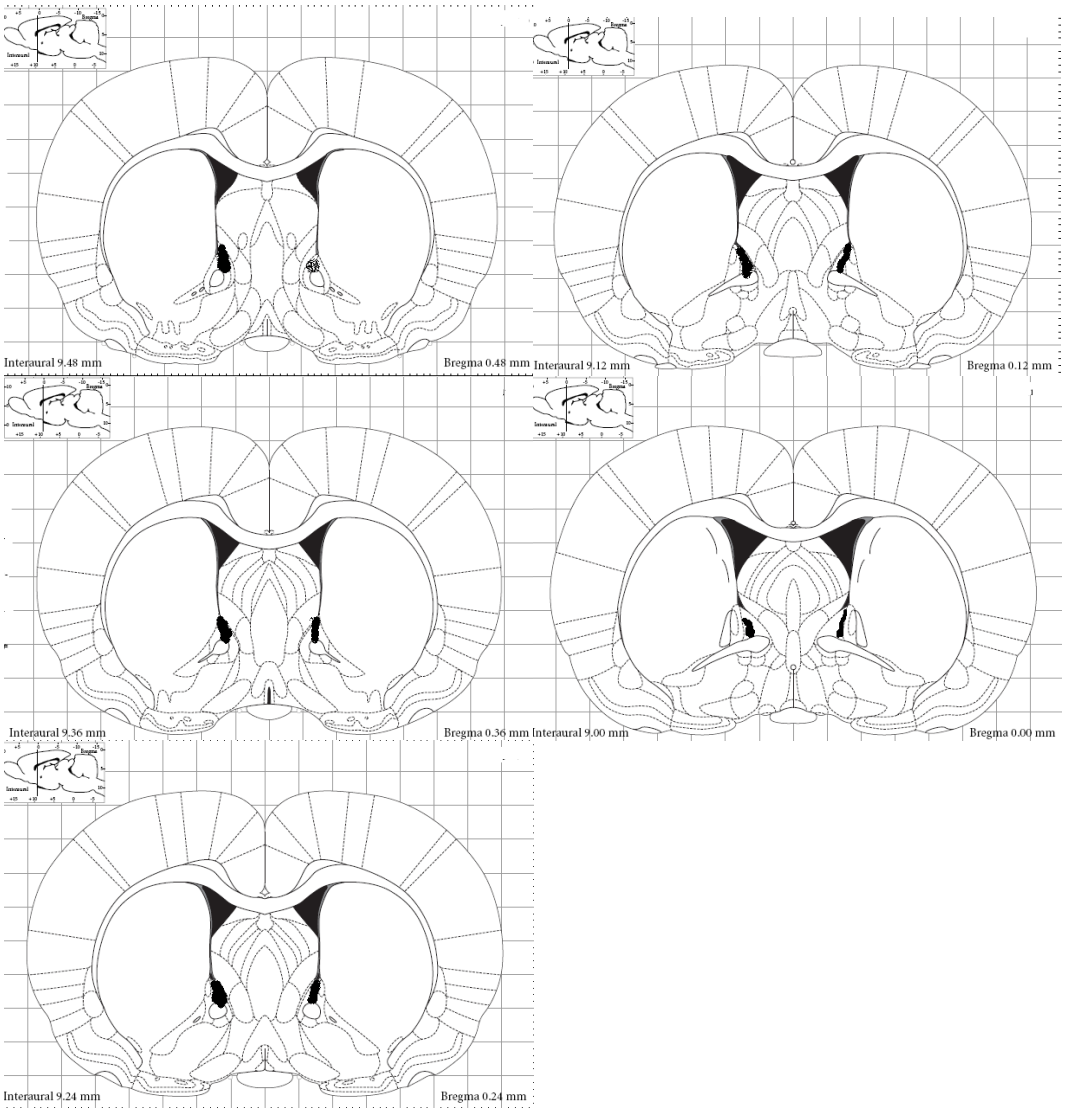



Figure 3: BNST lesion for the #35. Brain Maps are adapted from Paxinos and Watson, 1997

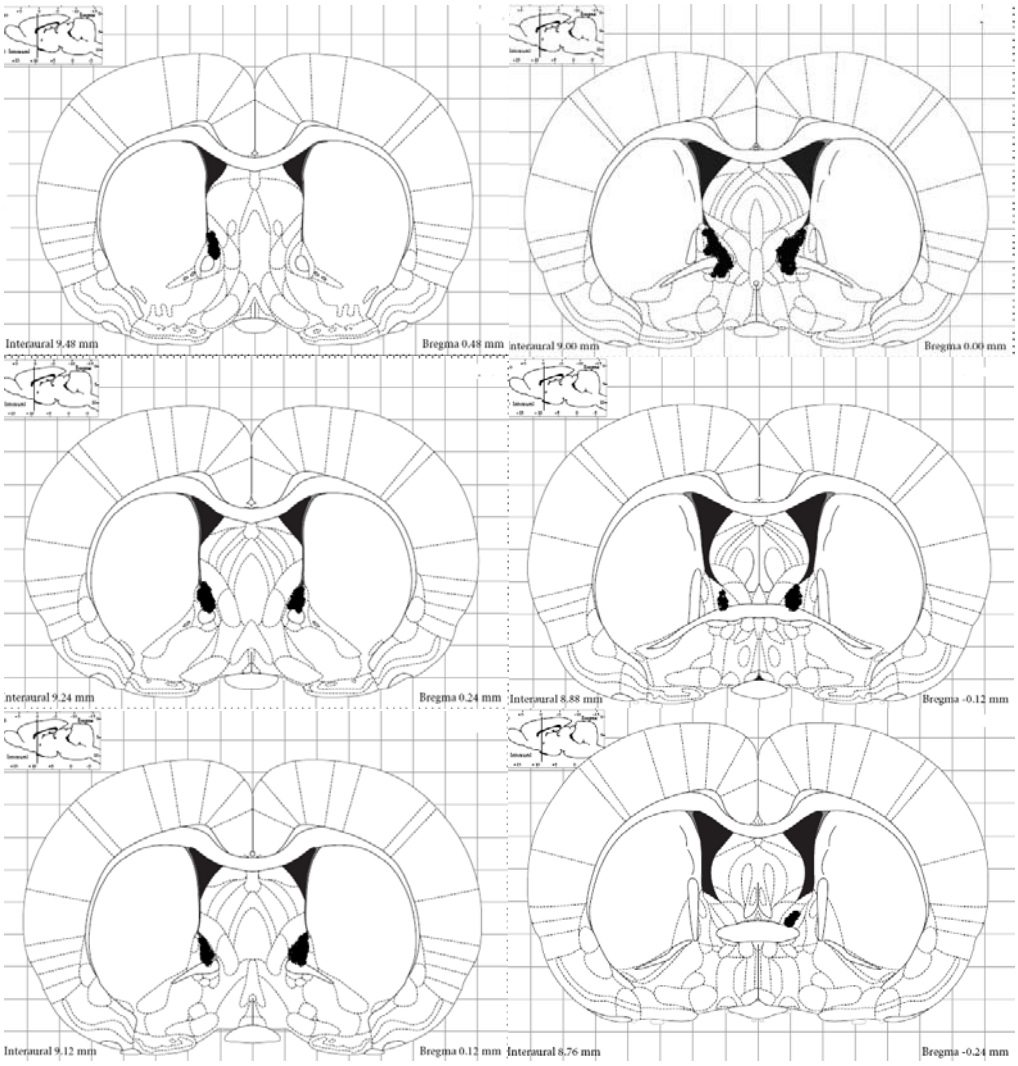



Figure 4: BNST lesion for the #37. Brain Maps are adapted from Paxinos and Watson, 1997

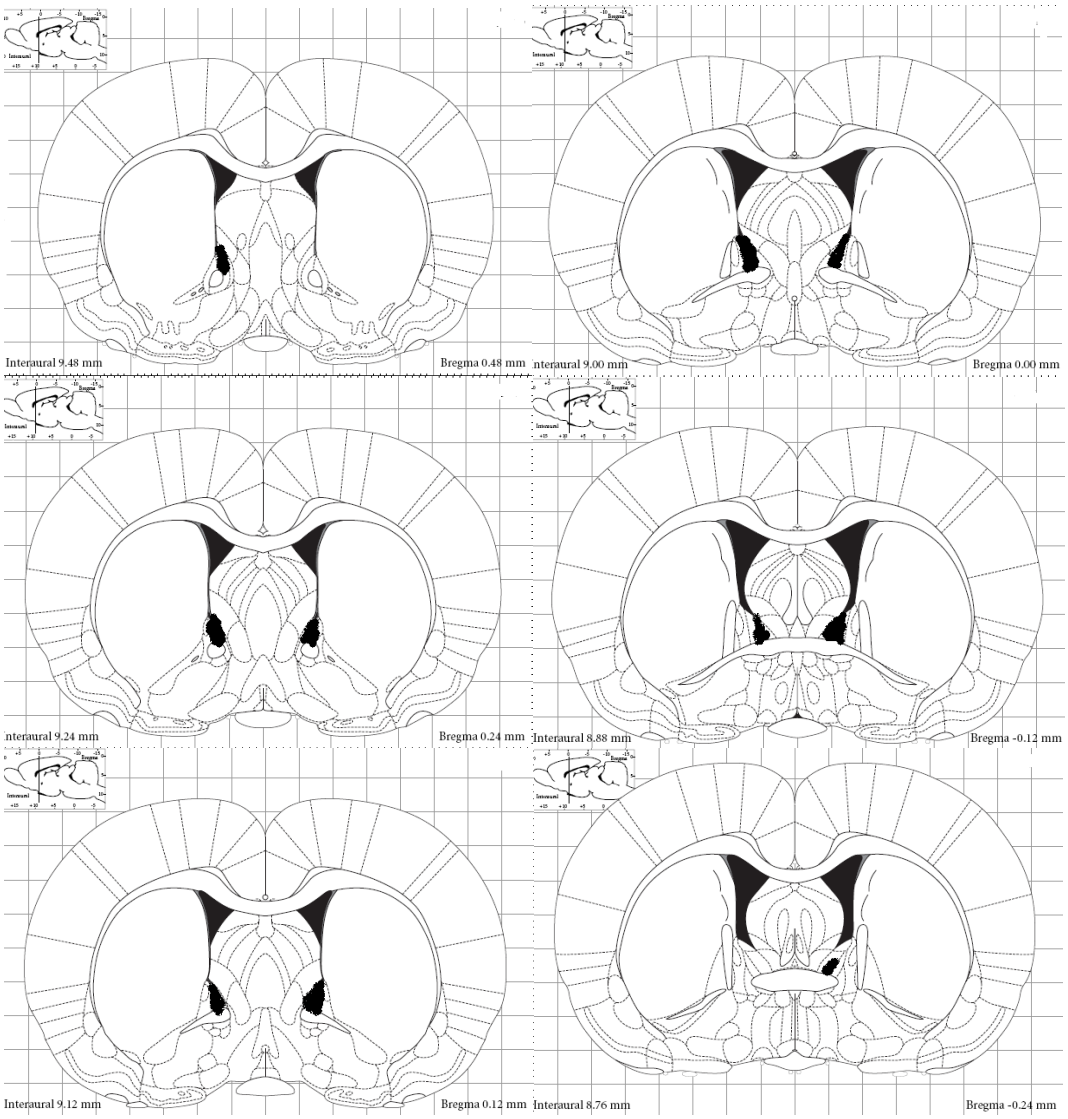



Figure 5: BNST lesion for the #42. Brain Maps are adapted from Paxinos and Watson, 1997

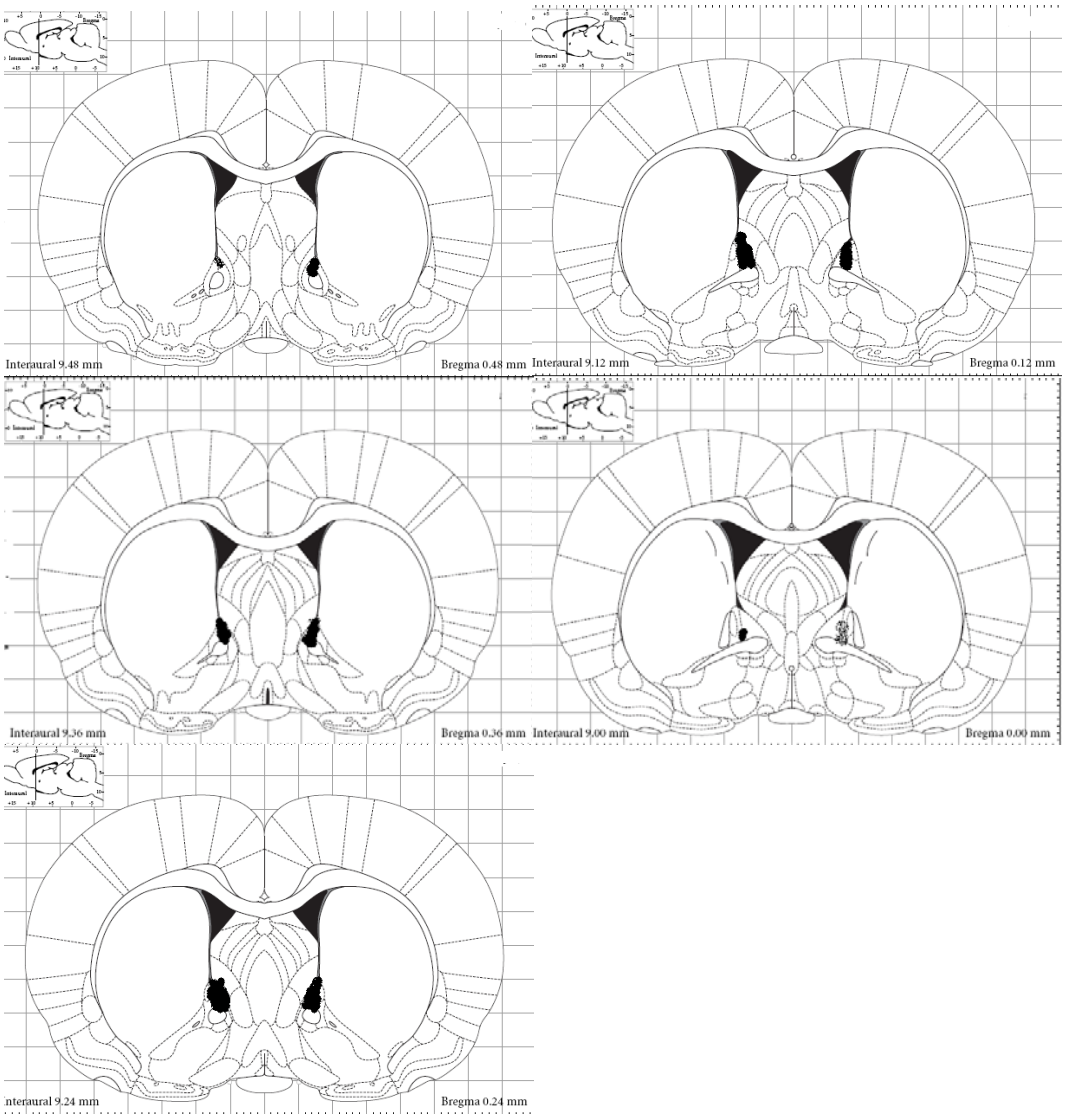



Figure 6: BNST lesion for the #52. Brain Maps are adapted from Paxinos and Watson, 1997

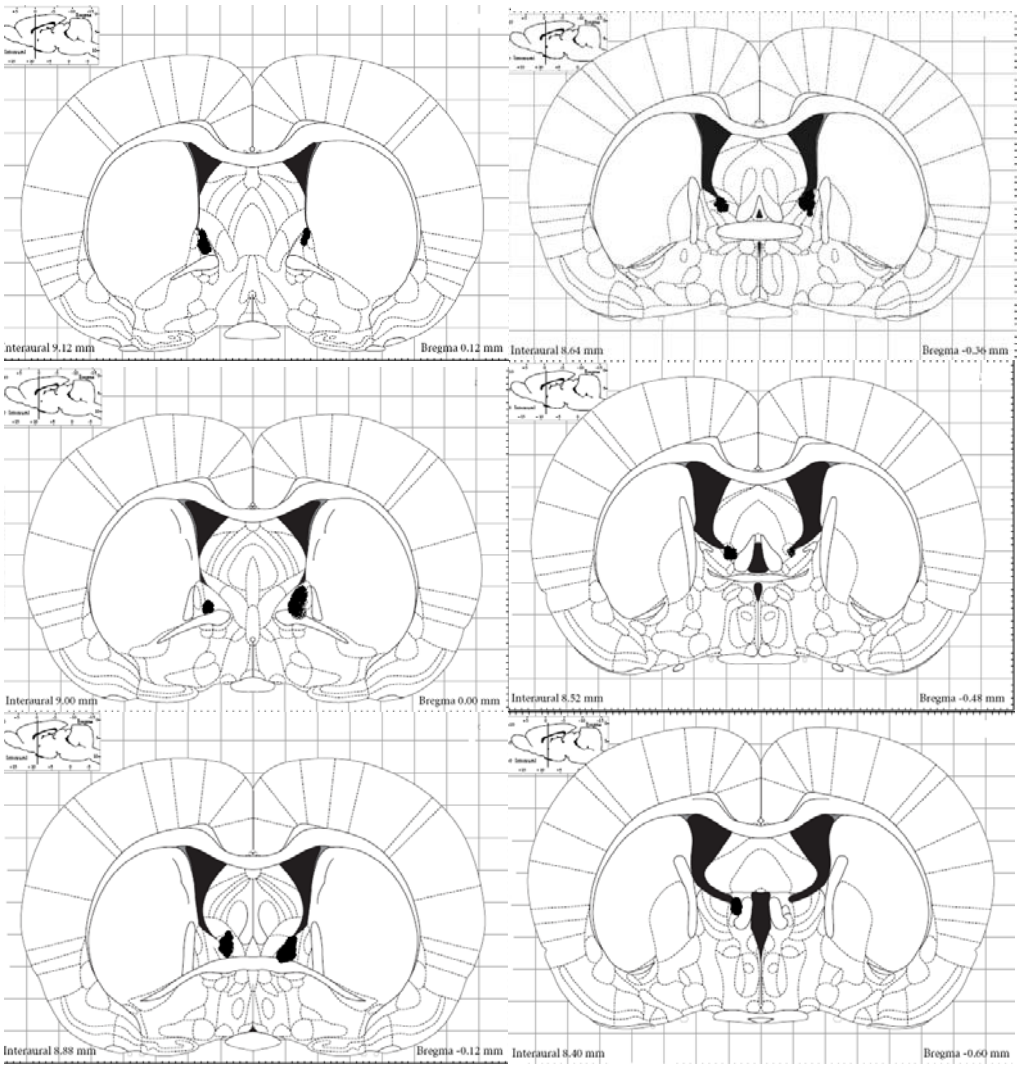



Figure 7: BNST lesion for the #56. Brain Maps are adapted from Paxinos and Watson, 1997

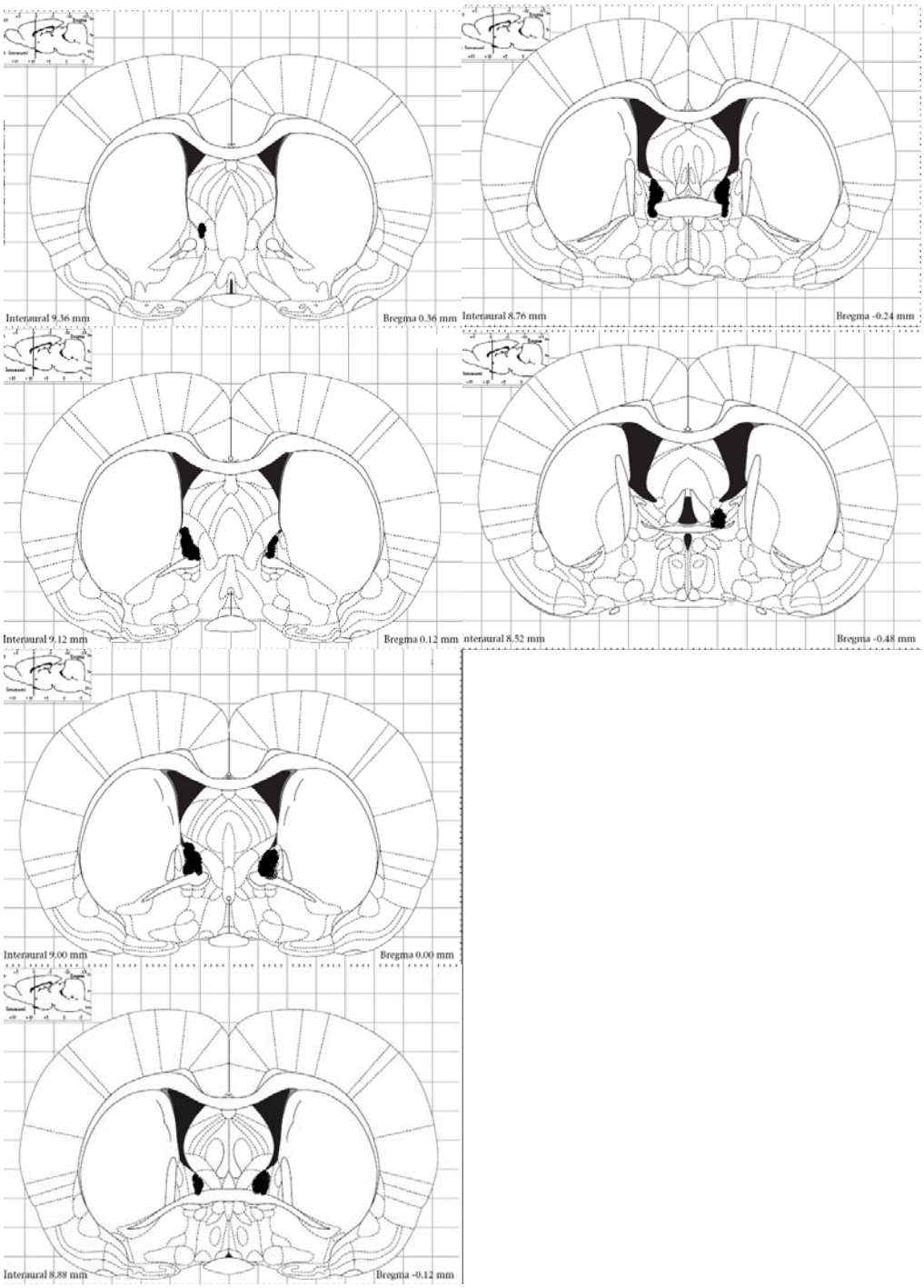



Figure 8: BNST lesion for the #60. Brain Maps are adapted from Paxinos and Watson, 1997

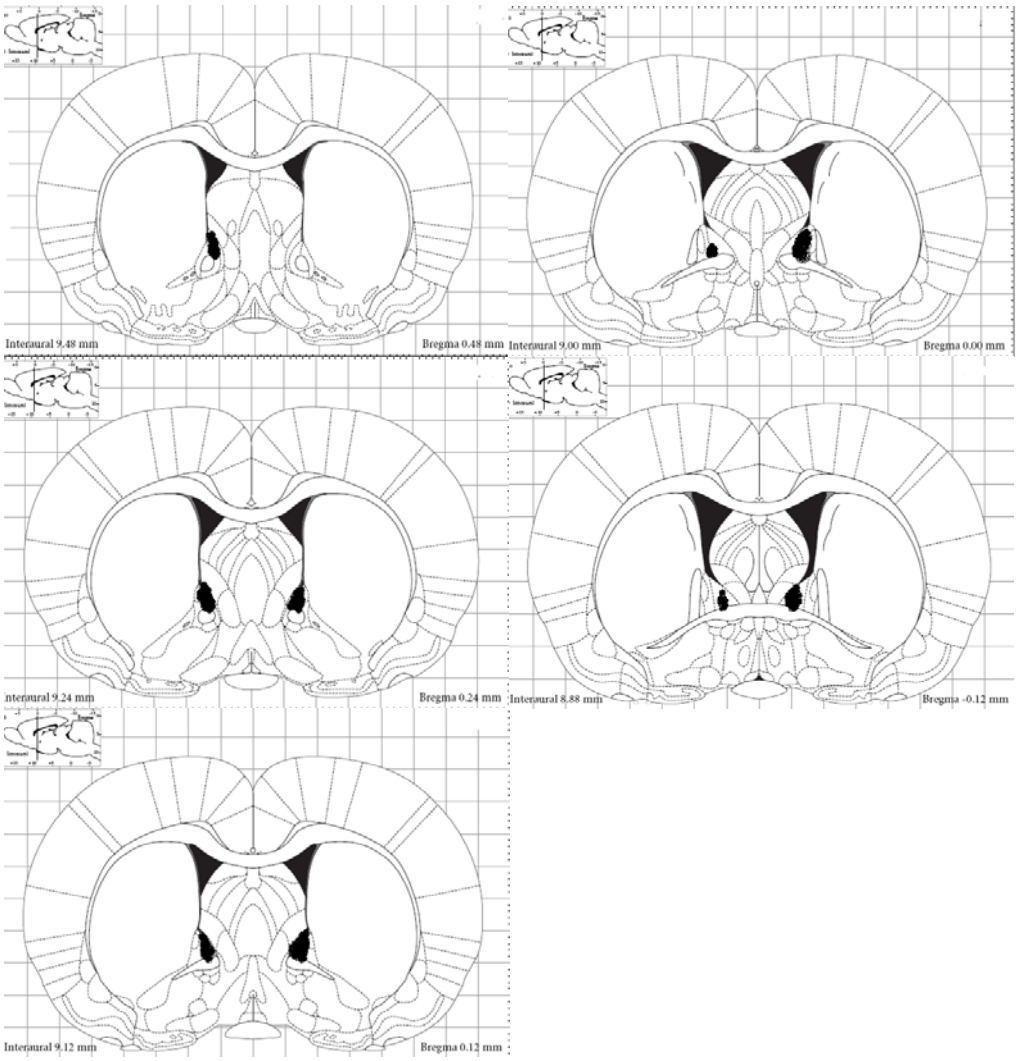



Figure 9: BNST lesion for the #63. Brain Maps are adapted from Paxinos and Watson, 1997

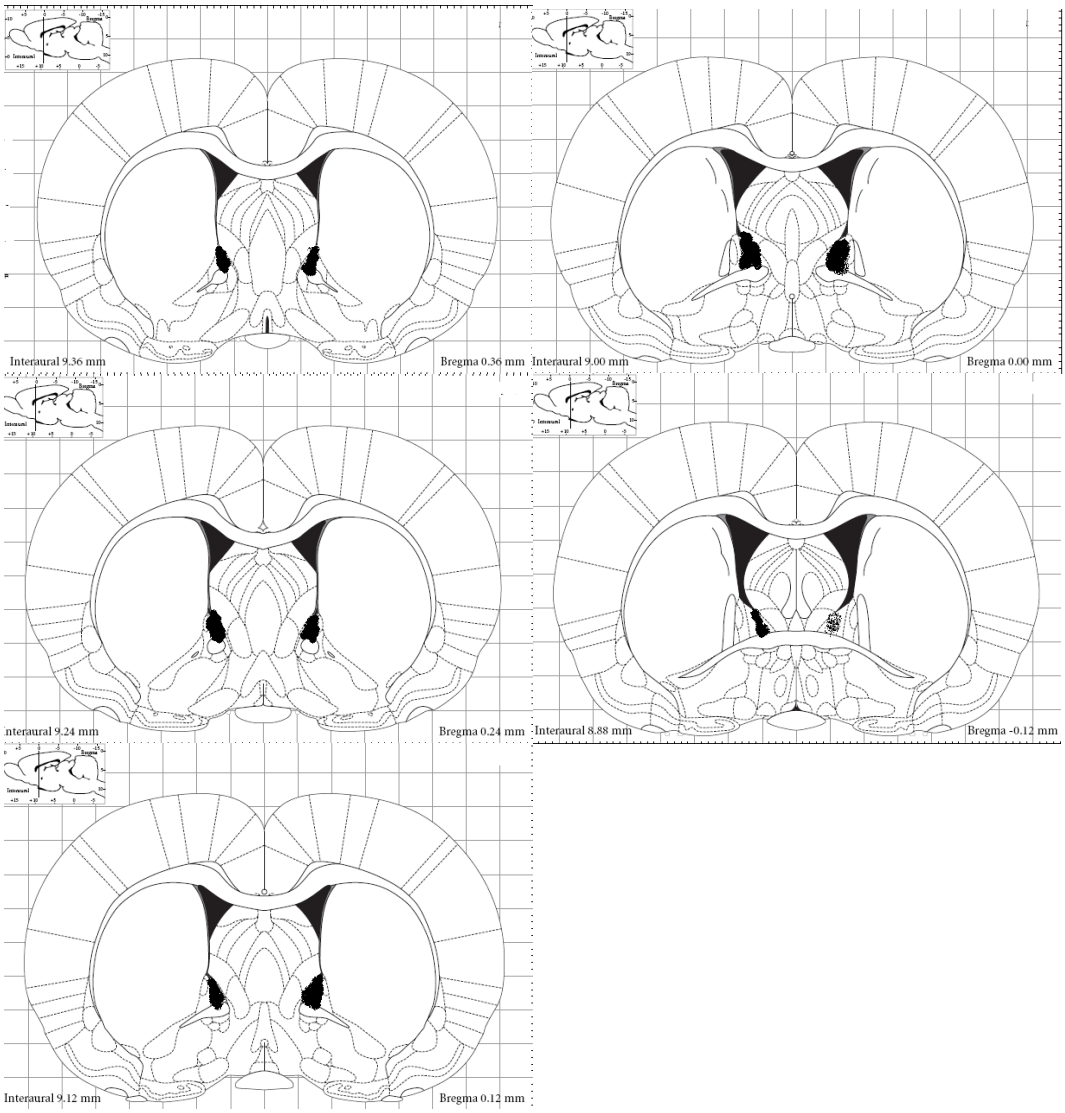



Figure 10: BNST lesion for the #70. Brain Maps are adapted from Paxinos and Watson, 1997

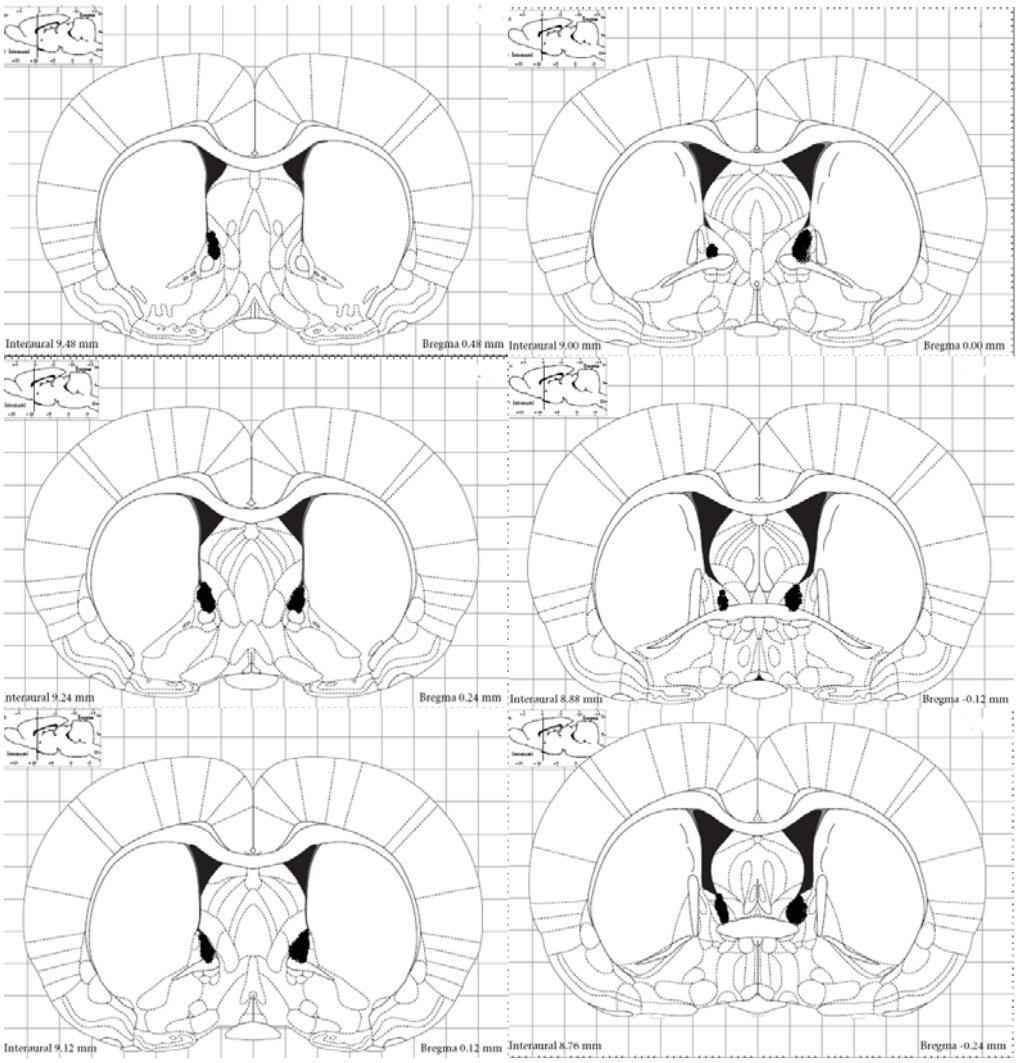



Figure 11: BNST lesion for the #71. Brain Maps are adapted from Paxinos and Watson, 1997

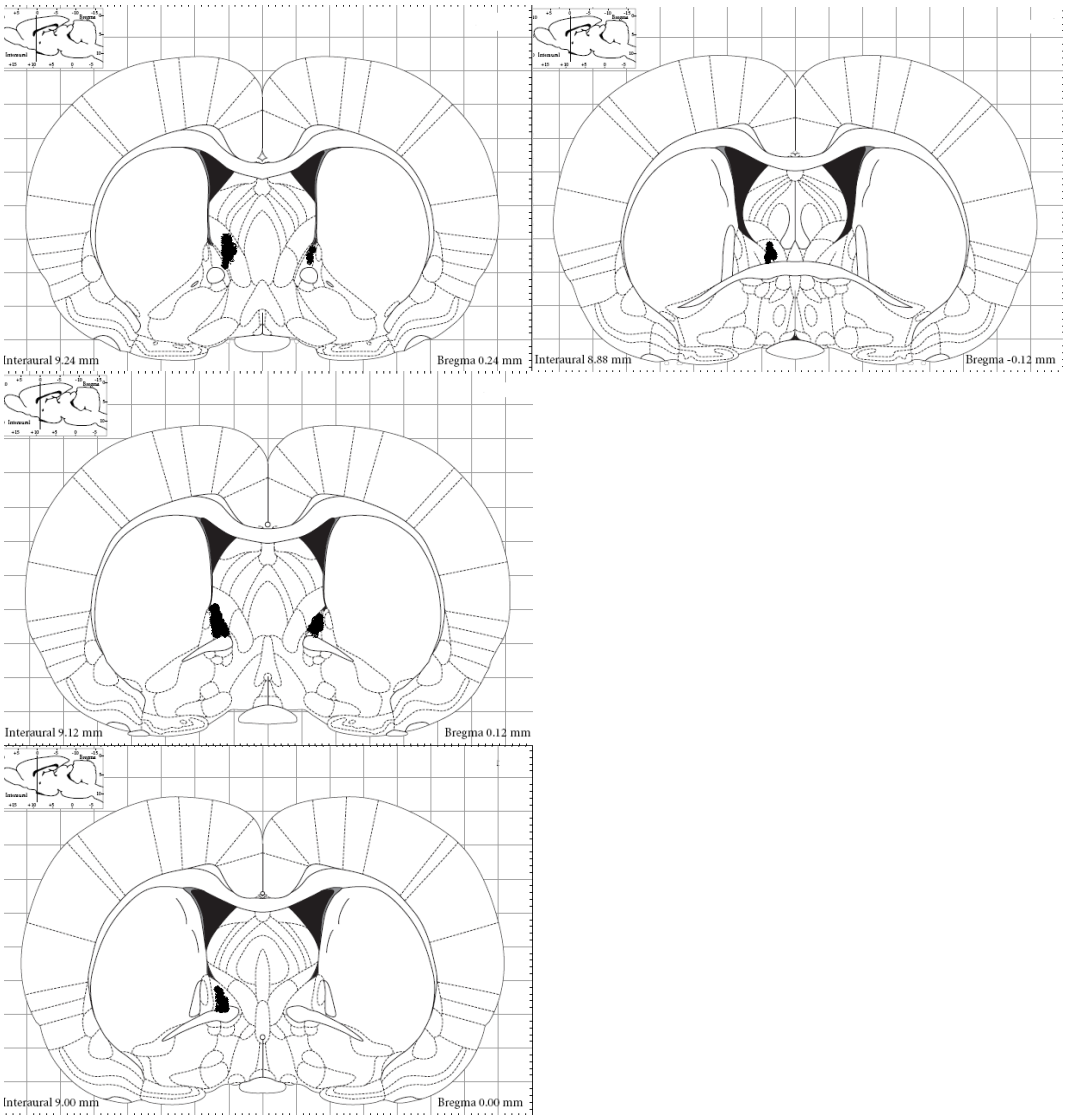



Figure 12: BNST lesion for the #75. Brain Maps are adapted from Paxinos and Watson, 1997

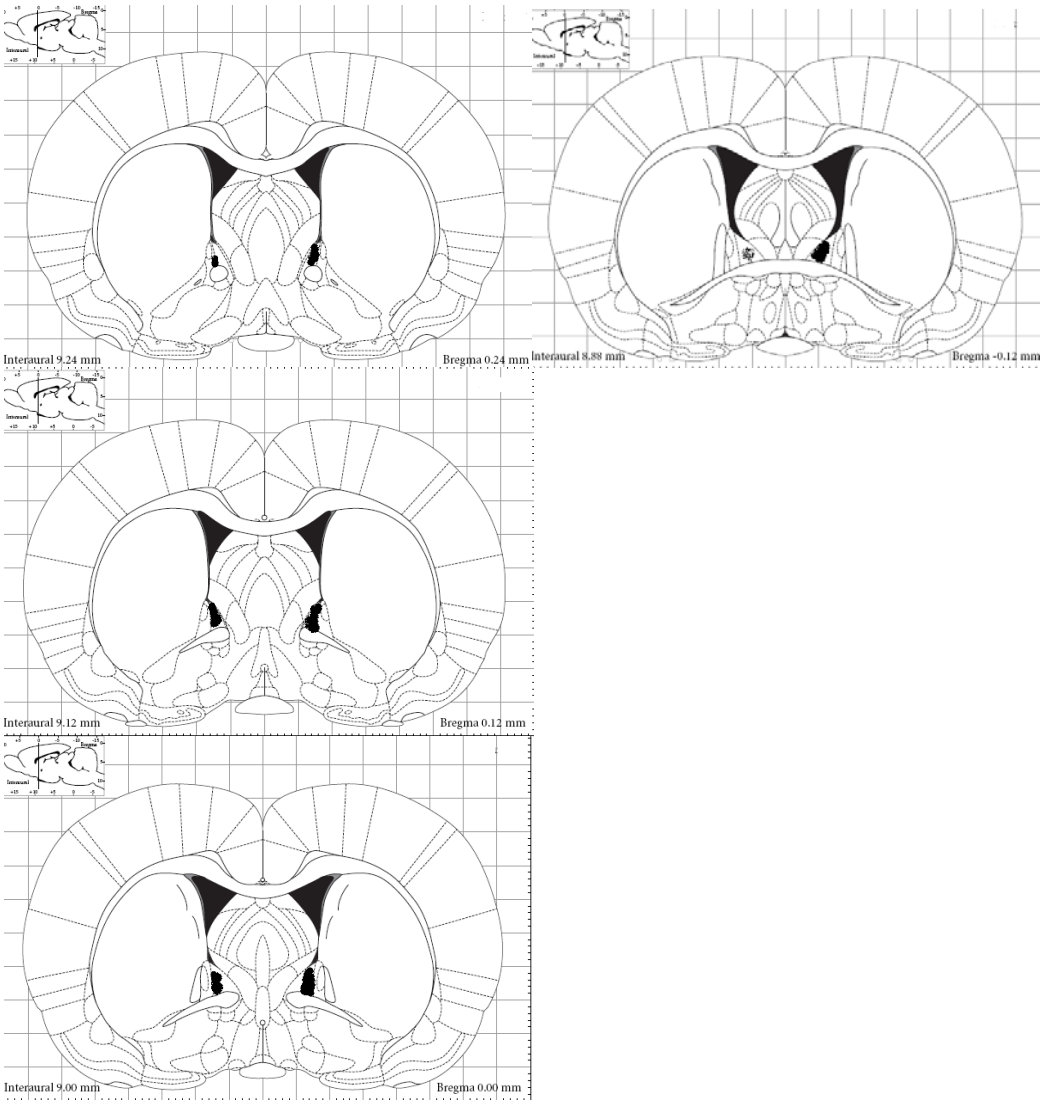



Figure 13: BNST lesion for the #80. Brain Maps are adapted from Paxinos and Watson, 1997

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