THE EFFECTS OF MS/VDB LESİONS ON BEHAVIORAL DESPAIR AND LEARNING AND MEMORY

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ABSTRACT

The present study aimed to investigate the consequences of hippocampal denervation in terms of irreversible medial septal area lesioning on behavioral despair and navigational learning. To that purpose medial septum / vertical diagonal band of Broca (MS/VDB) lesions were achieved electrolytically or with 980-nm diode laser application in the rat brain. The animals were tested in forced swim test followed a week later by Morris water maze to assess behavioral despair and navigational ability respectively.

Histochemical evaluation revealed lower acetylcholinesterase (AChE) content in the hippocampus of some of the lesioned animals compared to sham-operated control animals as a functional outcome of MS/VDB lesions. Animals with low AChE content in the hippocampus showed aggravated behavioral despair determined by augmented duration of immobility in the second swim test. On the other hand, the temporal learning acquisition in Morris water maze rather than total learning capability is affected by medial septal area lesions. Behavioral findings in the present study appear, not to be due to possible sensory-motor impairments of the lesioned animals since the latter did not differ from the sham-operated controls in the visible platform version of MWM task and open field activity test. Electrolytic lesions appear to be more efficient than laser lesions in terms of AChE decrease in the hippocampus.

In conclusion, reduction of hippocampal AChE content via irreversible lesions of MS/VDB area aggravates behavioral despair but fails to induce learning impairments in rats.

Keywords: 980-nm diode laser, electrocoagulation, neurosurgery, acetylcholinesterase, forced swim test, Morris water maze

MS/VDB LEZYONLARININ DAVRANIŞSAL ÇARESİZLİK VE ÖĞRENME VE BELLEK ÜZERİNDEKİ ETKİLERİ

ÖZET

Bu çalışmanın amacı, medyal septal bölgenin lezyonu ile ortaya çıkan hippokampal denervasyonun davranışsal çaresizlik ve uzamsal öğrenme üzerindeki etkilerini araştırmaktı. Bu maksatla medial septum / vertical diagonal band of Broca (MS/VDB) bölgesi sıçan beyninde elektrolitik ve 980-nm diyot laser ile lezyonlanmıştır. Hayvanlardaki davranışsal çaresizlik zorunlu yüzme testi ile uzamsal öğrenme ve bellek ise Morris su labirenti ile ölçülmüştür.

Lezyonlu hayvanlarda kontrol operasyonu geçirmiş hayvanlara göre hippokampal asetilkolinesteraz miktarında azalma görülmüştür. Hippokampüslerinde düşük asetilkolinesteraz miktarı görülen hayvanlar kontrollere göre ikinci yüzme testindeki artmış hareketsizlik süreleri göz önüne alındığında kötüleşmiş davranışsal çaresizlik göstermişlerdir. Medyal septal bölgenin lezyonu Morris su labirentindeki genel öğrenme kabiliyetinden ziyade günler içinde öğrenme sürecini etkilemektedir. Bu çalışmadaki davranışsal sonuçların olası motor bozukluklarından kaynaklanmadığı görülmektedir; Morris su labirentinin görünür platformlu denemelerinde ve açık alan testinde lezyonlu hayvanlar ile kontrol operasyonu geçirmiş olanlar arasında fark bulunmamıştır. Hippokampüsteki asetilkolinesteraz azalması göz önüne alındığında medyal septal bölgenin elektrolitik olarak lezyonlanu laser ile lezyonundan daha başarılı sonuçlar ortaya çıkarmıştır.

Özetle, MS/VDB bölgesinin kalıcı lezyonları sonucunda hippokampüste azalan asetilkolinesteraz miktarı davranışsal çaresizliği kötüleştirmekte ama öğrenme bozukluklarına yol açmakta yetersiz kalmaktadır.

Anahtar Sözcükler: 980-nm diyot laser, elektrokoagülasyon, sinircerrahisi, asetilkolinesteraz, zorunlu yüzme testi, Morris su labirenti

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LIST OF ABBREVIATIONS

μm	micrometer
AC	Alternating Current
Ach	Acetylcholine
AChE	Acetylcholinesterase
AMPA	α-**a**mino-5-hydroxy-3-**m**ethyl-4-isoxazole **p**ropionic **a**cid
ANOVA	Analysis Of Variance
AP	Anteroposterior
ATA	Ablated Tissue Area
CA1	Cornu ammonis 1
CA2	Cornu ammonis 2
CA3	Cornu ammonis 3
CA4	Cornu ammonis 4
CCP	Cell Counting Program
CFV	Cresyl Fast Violet
CGRP	Calcitonin gene-related peptide
ChAT	Choline acetyl transferase
CNS	Central Nervous System
CO_2	Carbon dioxide
CRH	Corticotrophin releasing hormone
CRH-R	CRH- receptor1
CW	Continuous wave
CZ1	Coagulated Zone One

CZ2	Coagulated Zone Two
DC	Direct Current
DV	Dorsoventral
Er:YAG	Erbium Yttrium Aluminum Garnet
FRL	Flinders Resistant Line
FSL	Flinders Sensitive Line
FST	Forced swim test
FST 1	Forced swim test 1
FST 2	Forced swim test 2
g	Gram
GABA	Gamma aminobutiric acid
Glu	Glutamate
GR	Glucocorticoid receptor
H&E	Hematoxylin and Eosin
High	AChE High-Acetylcholinesterase
HPA	Hypothalamo-pituitary-adrenal
HpD	Hematoporphyrin
i.c.v.	Intracerebroventricular
KTP	Kalium Titanyl Phosphat
LASER	Light Amplification by Stimulated Emission of Radiation
LHRH	Luteinizing hormone releasing hormone
LITT	Laser-induced interstitial thermotherapy
Low	AChE Low-Acetylcholinesterase
LSD	Least significance difference
mA	Miliamper
mAChR	Metabotropic ACh receptor

ML	Mediolateral
ml	Milliliter
MR	Mineralocorticoid receptor
mRNA	Messenger RNA
MS/VDB	Medial septum/vertical diagonal band of Broca
MSA	Medial septal area
MTP	Match-to-position
MWM	Morris Water Maze
nAChRs	Nicotinic ACh receptors
Nd:YAG	Neodmium Yttrium Aluminum Garnet
NGF	Nerve growth factor
nm	Nanometer
NMTP	On-match-to-position
OF	Open field
ORG 34116 (a substituted 11,21 bisarylsteroid compound)	
PB	Phosphate Buffer
PB	Phosphate buffer
PDT	Photodynamic Therapy
PFA	Paraformaldehyde
PFA	Paraformaldehyde
PVN	Paraventricular nucleus
RAM	Radial arm maze
RER	Rough Endoplasmic Reticulum
RNA	Ribonucleic Acid
RU 38486	(17/3-hydroxy- 1113-(4-dimethylamino-phenyl)17a-(1-propynyl)estra-4,9-diene-3-one))

RU38486	11beta-(4-dimethylaminophenyl)-17beta-hydroxy,-17a-(prop-1-ynyl)-estra-4,9-dien-3-one (“mifepristone”)
s	Second
SD	Standard deviation
W	Watt

1. INTRODUCTION

1.1 Motivation and Objectives

Brain lesioning methods have been widely used and generally accepted in investigating the specific roles of different brain regions on behavioral paradigms such as depression, learning and memory as well as motor activity. Current methodology involves mainly the applications of direct current, radio frequency as well as laser light directly into specific brain areas to induce ablation.

The present study has its grounds on our previous experiments from two points of view. Previous research has shown that 980-nm diode laser application is a suitable and valuable tool for neurosurgery as assessed by selective brain lesions in rats. The high water absorption coefficient of diode lasers prevents excessive scattering and leads to specific brain lesions with minimal thermal damage to adjacent tissue when compared to other conventional lasers. In addition, their light weight, portable size, lower cost, longer operating life and better operating conditions make the use of 980-nm diode lasers as valuable surgical tools. While, anatomical and histopathological outcomes of 980-nm diode laser lesions in rat brain have been reported in comparison with electrocoagulative lesions, 980-nm diode laser lesioned animals have not been used before for behavioral investigations.

In our investigation on the brain structures that are involved in the presentation of depressive states, the medial septal area appears to have an important role through its massive connections with hippocampal formation that is not only one of the main structures involved in learning and memory processes but also a modulator of affective states. In that respect the current study aims to reveal the possible involvement of medial septum/vertical diagonal band of Broca (MS/VDB) in behavioral despair as well. Indeed, our prior investigations have led to the conclusion that the absence of medial septum aggravates behavioral despair in female rats (unpublished data). Since the representation of behavioral despair is thought to differ between genders the necessity arises to demonstrate the effects of medial septum lesions in males as well. Although several studies implicate a

significant role of medial septum on learning and memory paradigms thought its extensive connections with hippocampal formation, differences in testing schedule as well as the testing apparatus might have contributions on cognitive outcomes. Thus, it has been found essential to investigate the effects of medial septum lesions on behavioral despair as well as navigational learning.

The present study was conducted to elucidate the role of the medial septal area ablation with two different tools (electrolytic or 980-nm diode laser) on behavioral despair followed by navigational learning.

1.2 Outline

In Chapter 2, background information is given about the current and laser beam use for brain tissue lesioning, location and connections of medial septal area, the role of medial septal area in affective and cognitive states.

Chapter 3 gives detailed information about the chemicals, solutions, and equipments used in this study.

In Chapter 4 the methods of stereotaxic neurosurgery, behavioral testing and histological examination are described.

The histological and behavioral results of the present study are available in Chapter 4.

The results of the present study are discussed in Chapter 5 while the general conclusions and future works are presented in Chapter 6.

2. BACKGROUND

2.1 The Use of Electrocoagulative Tool Versus 980-nm Diode Laser In Brain Tissue Lesioning

The common methods used in neurosurgery include electro-coagulation, radio-frequency and laser. Electro-coagulation is thought to exert its damaging effects either electrolytically or thermally [1, 2] whereas laser effects are photo-thermal by conversion of laser light into heat. So far Carbon Dioxide (CO_2) lasers have been proposed as useful tools for cutting brain tissue [3], Argon ion lasers for vascular tumors [4, 5], Neodmium Yttrium Aluminum Garnet (ND:YAG) laser for brain tissue and vessel coagulation that appears to be poorly effective due to irreversible and large thermal damage to nearby tissue [5] and Erbium Yttrium Aluminum Garnet (Er:YAG) lasers for brain tissue ablation [6].

In brain tissue coagulation the successful operating range of a particular laser depends mostly on the water absorption coefficient since approximately 70-80% of brain neuronal tissue appears to be water [7, 8]. Due to its comparatively high water absorption coefficient as well as light weight, portable size, lower cost, longer operating life and better operating conditions, 980-nm diode lasers have been proposed as valuable surgical tools [9]. Previous efforts administered the functional equality and qualitative benefits of 980-nm laser lesions over the electro-coagulative lesions [10-17]. In that respect dosimetry studies revealed that 2W-2sec (continuous wave 980-nm) is an optimum dose to create spherical lesions with diameter 2-3 mm in rat brain [10, 11]. On the other hand, post-surgical examination of 980-nm laser lesions in brain tissue appear to have healthier occurrence in recovery period as assessed by the quantification of macrophage density in the nearby area of the targeted brain region. Specifically, it has been shown that the most remarkable difference between electrocoagulator and laser lesions in the first 7-days after neurosurgery is the significantly higher accumulation of macrophages in the surrounding tissue of electrocoagulative lesions compared to 980-nm laser lesions [16, 17].

Although microglial activation is known to be a common feature of central nervous system lesions [18, 19], evidence suggests that the time-course and lesion type affect the proliferative activity of the microglia/macrophage response [20-23]. General agreement claims that microglia/macrophage activity peaks at about 5-7 days post-lesion and declines considerably after about 30 days [18]. Accordingly, brain lesions introduced electrocoagulatively and by 980-nm diode laser are thought to be alike after 40 days of surgery in terms of expected immune response.

2.2 The Location and Connections of MS/VDB

The medial septal area (MSA) is the most rostral part of the basal forebrain that is located between the anterior hippocampal rudiment and vertical limb nucleus, and is a vertically oriented roughly boomerang shaped nucleus [24]. Functionally MSA is usually considered together with the vertical limb of the diagonal band of Broca since there appears to be no anatomical boundary between the two and both structures are thought to be classified as separate nuclei somewhat arbitrarily [24]. Therefore, in the present context medial septum / vertical limb of diagonal band of Broca (MS/VDB) will be treated as a single functional unit.

MS/VDB neurons project primarily to the hippocampal formation via dorsal fornix, fimbria, the supracallosal striae and a ventral route through the amygdala [25] and to a lesser extend to the entorhinal cortex [26, 27]; additionally it has projections to the medial and lateral preoptic areas through the medial forebrain bundle. Furthermore, fibers originating from MS/VDB have been shown to extend to the midbrain and to the parataenial nucleus of the thalamus [24].

2.2.1 The Hippocampal Connections of MS/VDB

The most prominent connection of MS/VDB is considered to be the cholinergic innervation of hippocampal formation that constitutes about 40-50% of the medial septum neurons whereas projections that are GABAergic (gamma aminobutyric acid) in nature

involve 10-20% of the MS/VDB cells [28]. Recent investigations have uncovered the existence of glutamatergic neurons located in MS/VDB that project to the hippocampal formation as well and represent 23% of the septo-hippocampal projection [29]. Acetylcholine (ACh) has been proposed to exert fast excitatory and a slower modulatory effect on hippocampal excitability [30], GABAergic projections, on the other hand, lead to a net excitability by the inhibition of the inhibitory GABAergic cells in the hippocampus [31]. Glutamatergic neurons appear to constitute a local excitatory network that excites both cholinergic and GABAergic neurons within MS/VDB complex [32] and await further attention for the elucidation of their function in the hippocampus.

Additionally, several peptides have been shown to be localized in the efferent neurons of MS/VDB such as galanin, calcitonin gene-related peptide (CGRP) and luteinizing hormone releasing hormone (LHRH); among these, galanin-positive cells, co-localizing mostly with cholinergic ones [33], appear to have the highest mass with presence in the 22% of neurons [34]. Nerve growth factor receptors have been shown to co-exist in about 70% of choline acetyltransferase containing cells of MS/VDB [35]. Although the role of Nerve growth factor (NGF) receptors located in MS/VDB has been thought to be essential for the survival of cholinergic neurons [36], evidence suggests that these receptors are likely to modulate the enzymatic activity of choline acetyltransferase (ChAT) [37]. Substance P [38], somatostatin, neurotensin [39] and parvalbumin [28] are among other peptides localized in the medial septum.

2.2.2 Other Connections of MS/VDB

Although the projections of MS/VDB have been mostly implicated to be hippocampus-related, there is evidence that MS/VDB has a direct projection to the amygdala [40, 41] and paraventricular nucleus (PVN) [42]. Other targets of MS/VDB appear to be the cholinergic projections to the cingulate and occipital cortices and to the olfactory bulb [43, 44].

2.3 The Histochemical Outcomes of MS/VDB Lesions

Importantly, cholinergic projections originating from MS/VDB supply about 90% of the cholinergic innervation of hippocampal formation underlying further the importance of MS/VDB integrity for the proper functioning of the hippocampal formation. Many studies indicate that MS/VDB lesions regardless of the technique involved (e.g. electrolytic or chemical lesion) lead to depletion in the acetylcholinesterase (AChE) levels in the hippocampus. However, an interesting study conducted by Oderfeld and Potempska [45] has noted that the location, the size as well as the extension of the electrolytic septal lesions determines the AChE level in hippocampus in a time dependent manner. Specifically, total destruction of septal nuclei represents itself as an 80% decrease in hippocampal AChE activity that is evident after the fifth day of lesion and does not recover as examined after a year. However, extensive medioventral lesions of septal area appears to lead to temporal 70% decrease in AChE activity that is recovered up to 40% in 30 days, whereas a small medioventral damage results in a persistent 40% decrease in AChE activity in hippocampus. The authors suggest possible sprouting of the cholinergic nerve terminals from intact parts of the septal area [45].

2.4 The Role of MS/VDB In Affective States Specifically Depression

Major depression is mostly a recurrent lifelong illness that is thought to affect about 8% of men and 15% of women throughout life [46]. Disturbances of the hypothalamo-pituitary-adrenal (HPA) axis have been proposed to play a significant role in the initiation as well as recurrence of depression [47, 48].

The HPA axis is usually involved in the sequence of events that starts with the effect of stress on hypothalamic paraventricular nucleus leading to secretion of corticotrophin releasing hormone (CRH) that subsequently activates the pituitary gland and results in adrenocorticotropic hormone (ACTH) secretion. Subsequently, ACTH increases

glucocorticoid levels (e.g., corticosterone in rats, cortisol in humans) by acting on the adrenal glands (Figure 2.1). Evidence from human studies reveals that depressed patients have elevated cortisol concentrations in their plasma, cerebrospinal fluid and urine [49]. Antidepressant treatment of depressed patients appears to restore HPA axis activity [47].

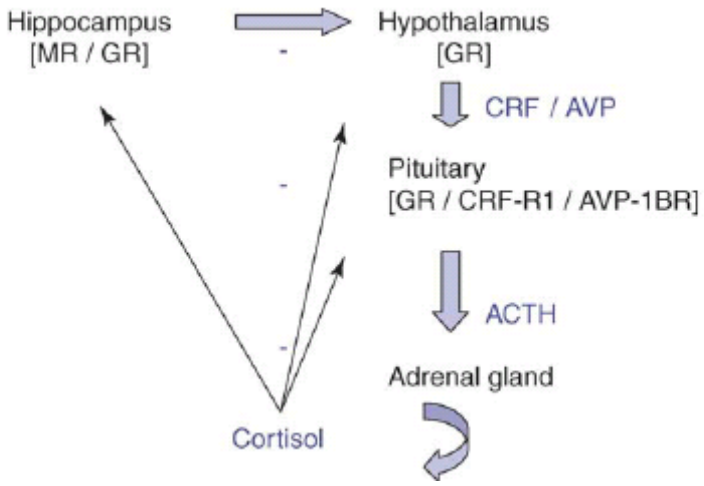


Figure 2.1 The schematic representation of the hypothalamo-pituitary-adrenal (HPA) axis and inhibitory feedback mechanism of cortisol [46].

On the other hand, studies involving animal subjects indicate that forced swim test (FST) is a reliable test for the evaluation of antidepressant actions [50, 51]. Moreover, it has been shown that reduction in the corticosterone concentrations by adrenolectomy [52], administration of glucocorticoid antagonists RU38486 (11beta-(4-dimethylaminophenyl)-17beta-hydroxy,-17a-(prop-1-ynyl)-estra-4,9-dien-3-one) [53, 54], ORG 34116 (a substituted 11,21 bisarylsteroid compound) [55] or corticosterone synthesis inhibitor [56] lead to lowered immobility duration in the forced swim test. On the other hand corticosterone administration returns the immobility to a higher level that is lowered by adrenolectomy [52]. Additionally, a recent study by Johnson et al. (2006) demonstrated that daily corticosterone administration of 40mg/kg for 21 consecutive days (but not for a single day) increases immobility and decreases the active behavior in forced swim test. Forced swim test itself has been shown to cause increase in plasma corticosterone levels [57, 58, 59] as well as CRH gene expression [60]. Thus, HPA axis integrity as well as feedback regulation appear to be important in the behavioral outcomes assessed by forced swimming in rats.

The literature so far lacks specific reports on the possible role of MS/VDB complex lesions on depression and/or depressive like states.

2.4.1 Effects of The Hippocampus On The HPA Axis

One of the regulations on HPA axis is directed via the hippocampal formation that has been thought to exhibit inhibitory effects [46, 61] (Figure 2.1). Damage to the hippocampus has been considered to cause further activation of HPA axis by disinhibition of the glucocorticoid negative feedback leading to subsequent elevation in glucocorticods (the "glucocorticoid cascade hypothesis") and possible neuronal degeneration [62]. Thus, loss of hippocampal function appears to result in HPA axis hypersecretion.

In that respect, large electrolytic septal lesions, which include both medial and lateral parts, appear to augment the corticosterone response to stressful stimuli such as exposure to a novel environment, noise, ether vapor or cold water, implicating the inhibitory effect of septal area on the corticosterone response to stressful events [63]. On the other hand electrical stimulation of medial septum as well as of the dorsal or ventral hippocampus has been shown to lead to increased plasma corticosterone level [64, 65].

Primary evidence for the involvement of the septohippocampal formation in HPA response to stress arises from the findings of the dense expression of mineralocorticoid (MR) as well as glucocorticoid (GR) receptors in hippocampal and septohippocampal neurons [54, 66-69]. A number of studies have elucidated the role of these two receptor types in the regulation of the HPA axis by hippocampus. The current view points towards differential functions of MR and GR. Namely, activation of MRs that are extensively occupied even in the presence low levels of glucocorticoids has been suggested to maintain the hippocampal excitability that would be expected to transmit inhibitory signals to PVN via GABAergic tones [70]. On the other hand, activation of GRs appears to suppress the hippocampal output that in contrast to MR activation would result in disinhibition of HPA axis. However, GR activation in PVN itself appears to have noticeable influence in the HPA axis inhibition [64].

Several studies reveal the effect of behavioral manipulations on the MR and GR levels. It has been shown that infusion of antisense oligonucleotides targeting glucocorticoid receptors in hippocampus as well as glucocorticoid receptor antagonist RU

38486 (17/3-hydroxy- 1113-(4-dimethylamino-phenyl)17a-(1-propynyl)estra-4,9-diene-3-one)) leads to decrease in immobile behavior in forced swim tests [53]. A detailed study by Gesing et al. (2001) has shown that MR levels at hippocampal CA1, CA2 and DG subregions is increased after a 15 min single session of forced swim test, novelty stress or psychological stressor but not cold exposure. The effect appears to have subregional and temporal specificity since increase of MR in CA2 and CA3 regions is observed after 8hr and in all regions after 24hr with return to the baseline values after 48hr of forced swim test exposure. Furthermore, this effect has been shown to be mediated by CRH since blockade of CRH receptors 10min prior to FST prevents the MR increase in hippocampus [68]. Expression of CRH itself appears to be activated in the hippocampus following forced swim test and can be reversed by antidepressant treatment [60]. Additionally, a single FST experience leads to an enhancement in the MR-mediated inhibitory control of the HPA axis within 24hr that is assessed by MR antagonist challenge [68]. The effect appears to be MR specific since no change has been observed in GR levels. An interesting evidence of no change in MR messenger RNA (mRNA) as a response to forced swim test indicates that there might be important differences at the transcriptional and translational control of MR and GR after exposure to stressful conditions [68]. However, findings of Drossopoulou (2004) indicated a slight increase in GR in the hippocampus 24hr after the second swim test [59] that might indicate the differences between the outcomes of the first swim test compared to the second one. Overall evidence appears to support the inhibitory actions of hippocampus on HPA axis that is mediated by increase in the MR level due to higher affinity to glucocorticoids.

Besides the behavioral findings, direct manipulations of brain structures reveal further the regulative mechanisms involving the glucorticoid receptors. Namely, electrolytic medial septum lesions have been shown to increase MR and GR mRNA expression in subregions of the hippocampus [66]. Briefly, MR mRNA appears to be upregulated in all hippocampal regions, being highest in the Cornu Ammonis 1 (CA1) region whereas increase in GR mRNA is detected in the dentate gyrus, CA1 and Cornu Ammonis 2 (CA2) but not in Cornu Ammonis 3 (CA3) and cornu ammonis 4 (CA4) [66]. Furthermore, medial septum lesions accompanied by serotonergic depletion have been shown not to affect MR mRNA but to increase GR mRNA selectively in CA1 and CA2 regions of the hippocampal formation [71]. However, conflicting results are reported in the case of specific acetylcholine denervation of hippocampal formation via 192 IgG-Saporin

infusions into MS/VDB. Apparently GR mRNA is found to be decreased but MR mRNA is unaffected in the hippocampus following lesions of the cholinergic input [72]. On the other hand, MR transcription appears to be regulated by mineralocorticoids in a specific spatio-temporal manner. Application of low doses of aldosterone that belongs to mineralocorticods family results in high levels of MR but not GR mRNA after 4hr in medial septum that is drastically lowered after 24hr, whereas GR mRNA levels in hippocampus after 8hr is increased in adrenalectomized rats [69].

The overall picture indicates the complexity of the hippocampal feedback modulation on HPA axis at the level of MR and GR expression. Medial septum appears to have a striking role in terms of its dense connections with hippocampal formation. However, the studies are pointing to the activation of diverse mechanisms according to the duration and type of the stressors.

2.4.2 Acetylcholine Functions In The HPA Axis

There is evidence that stimulation of the hippocampal cholinergic system, by injection of AChE inhibitor neostigmine elevates plasma ACTH and c-fos expression in PVN. An increase of acetylcholine thus appears to excite the HPA axis. In parallel the systemic infusion of acetylcholine agonists as well as AChE inhibitors appears to cause depressive mood [73, 74]. Additionally it has been shown that a single one-day forced swim test leads to an increase in hippocampal AChE RNA after 50 min that implies an activation of a mechanism to compensate for the hippocampal ACh release in response to a stressful event [75]. Another study has shown that the specific activity of AChE is lowered in the hippocampus after acute or chronic immobilization stress [76] that might either indicate a response of lowered ACh supply or most probably a mechanism functioning to increase the available ACh in response to stress. However, Das et al. (2005) have not mentioned anything about the interval between the stressful events and the analysis of enzyme activity that might be important in terms of the temporal changes after stress. Although different stress paradigms appear to evoke different activation routes, an increase in AChE mRNA following forced swim test might be due to decrease of AChE activity as well as increase of ACh content.

Another possible mechanism is the direct activity of CRH on hippocampal ACh release. As examined in the rat brain, intracerebroventricular (i.c.v.) CRH injection appears to cause increased hippocampal ACh release [77] that is possibly mediated by septohippocampal neurons since i.c.v. CRH injections have been demonstrated to result in moderate to strong stimulation of Fos expression that is co-localized with CRH- receptor1 (CRH-R1) mRNA in MS/VDB [78].

The involvement of acetylcholine in affective states is further implied in the studies conducted with different rat strains. For example, reduction of choline uptake and elevation of muscarinic binding has been reported to occur after stress in Wistar Kyoto rats that are known to be more reactive to stress compared to their counterparts Brown-Norway rats that further suggests that the elevation of ACh is related to stress [79]. A more detailed research has been done on Flinders Sensitive Line (FSL) of rats that exhibit higher sensitivity to cholinergic agonists via elevated metabotropic ACh receptor (mAChR) numbers mainly in striatum and hippocampus but not the cortex [80, 81]. Chronic treatment with scopolamine or amitriptyline, which are anticholinergic drugs, as well as no treatment in FSL rats have been shown to increase immobility durations in forced swim test compared to Flinders Resistant Line (FRL) rats [80]. In parallel, studies in human subjects imply the involvement of supersensitivity to cholinergic drugs in the presence of depressive symptoms [82] and as a marker of history of depression [83]. The discrepancy between the two hypotheses remains unresolved but current approach points to the role of cholinergic supersensitivity towards increased risk for development of depression [81].

In addition to muscarinic acetylcholine receptor involvement, current studies indicate engagement of nicotinic acetylcholine receptors in the pharmacological treatment of depression as well. Specifically nicotinic AChR antagonist mecamylamine appears to have antidepressant effect as assessed by forced swim test; on the other hand mice lacking high-affinity nicotinic ACh receptors (nAChRs) have been shown to resist the antidepressant actions of the anticholinergic drug amitriptyline [84] that supports the role of cholinergic sensitivity in depression.

Antidepressants such as flurazepam, midazolam, and diazepam have been identified to act in an inhibitory manner on the spontaneous bursting activity of identified septohippocampal neurons [85]. This further implies the functional role of MS/VDB in handling depression.

2.5 The Involvement of MS/VDB In Learning Paradigms

2.5.1 Electrocoagulative, Radio Frequency and Aspiration Lesions

Although there is a consensus that disruption of MS/VDB region leads to severe learning and memory deficits, a detailed review of the literature reveals that the observed impairments depend on the task and the strategy used by the subjects [86]. In a study by Kelsey and Landry (1988) the authors point to the requirement of intact MS/VDB region for spatial mapping; they have shown that actually MS/VDB lesioned rats are impaired but capable of improving their performance throughout the training in Morris Water Maze (MWM) but are deficient in choosing the most accurate pathway that would lead to the hidden escape platform [87]. Similarly medial septum lesions via radiofrequency application cause impairments in strategy selection rather than total reference memory deficiency [88].

Septal lesions impair performance in radial arm maze (RAM) in postoperatively [89] and preoperatively trained rats [90]. Moreover, animals with septal lesions appear to have intact non-spatial memory but impaired spatial memory as assessed by delayed non-matching-to-sample task in a Y-maze [91].

Animals with MS/VDB lesions were shown to develop different strategies to solve spatial tasks. It has been reported that in a radial arm maze (RAM) task, subjects with MS/VDB lesions tend to switch from nonstereotypic to stereotypic strategy, that is the animals that use distal cues (e.g. solving the task without using a consistent response pattern) begin to use consistent response pattern (e.g. sequential entry of adjacent arms) after MS/VDB lesions [86]. However, another study with a similar but extended approach points to the deficiency of MS/VDB lesioned animals in using nonstereotypic strategy when they are forced to do so [92]. In a different spatial task that requires sequential discrimination of different locations, medial septum lesioned rats have been shown to be impaired [93].

2.5.2 Chemical and Immunotoxic Lesions

Ibotenate lesions of MS/VDB deplete acetylcholinesterase and ChAT in the hippocampus and impairs learning in MWM [94], radial arm maze (RAM) and passive avoidance task but not active avoidance [95]. Quisqualic acid lesions impair acquisition in MWM [96, 97] and conditional visual discrimination [97].

However, NMDA lesions of the MS/VDB region that also lead to severe depletion of AChE and AChT in the hippocampus and MS/VDB do not appear to induce memory impairment at the reference as well as working memory version of MWM (4trials/5days) or RAM [98]. Similarly, AMPA excitotoxicity in the medial septal area results in minor impairments in MWM [99].

Although neurotoxins such as ibotenic acid, quisqualic acid and AMPA (α-**a**mino-5-hydroxy-3-**m**ethyl-4-isoxazole **p**ropionic **a**cid) display some selectivity among neuron types it appears to be limited and more likely non-specific [100, 101]. In that respect, 192 IgG-saporin gains importance since it is a selective toxic substance for ACh neurons composed of an antibody against p75 nerve growth factor receptor and ribosome-inactivating protein saporin [102]. Evidence reveals that near complete lesion of the septohippocampal cholinergic projections via intraseptal 192 IgG-saporin infusions does not induce reference memory impairment in MWM [44, 102-104], RAM [105], inhibitory avoidance, non-match-to-position (NMTP) and match-to-position (MTP) T-test [104]. The failure of specific cholinergic denervation to cause learning and memory impairments has been mentioned in many reports [106, 107] and is thought to be attributed to the residual acetylcholine capacity of the hippocampal formation [108]. On the other hand, reports that reveal impairments in learning and memory paradigms are not missing from the literature of septal IgG-Saporin lesions [108, 109].

In addition to cholinergic mechanisms, GABAergic involvement should be considered in relation to learning and memory as well. In that respect kainic acid and/or saporin administration into the medial septum has been characterized to affect GABAergic and cholinergic neurons but fail to induce spatial learning impairments in MWM and RAM unless used in conjunction [110]. On the other hand, stimulation of the septal GABAergic neurons via muscimol appears to cause impairment in working memory tasks such as

spontaneous alternation that is reversed by acetylcholinesterase administration into the hippocampus [111].

Overall understanding of the current findings concerning the role of medial septal area on learning and memory paradigms points to a composite picture that depends on the lesioning method and the extend of lesions as well as the demands of the task and training duration.

3. MATERIALS AND METHODS

3.1 Subjects

Male Wistar rats (10 months of age) raised in the breeding colony of the Psychobiology Laboratory, weighing between 300-330 g at the start of the experiment, were randomly assigned to laser lesion, electrolytic lesion, and electrode-sham or fiber-sham groups. Fourteen rats were lesioned electrolytically and another 14 were lesioned with 980-nm diode laser, eight rats were sham-operated with electrode insertion and another eight rats were sham-operated with fiber insertion. All animals were maintained in a temperature controlled room (22±2 °C) on a 12h light/12h dark cycle (lights on at 08:00h). Animals were group-housed (6 per cage) with food and water ad libitum.

3.2 Stereotaxic Neurosurgery

All the surgical procedures were performed under 100mg/kg ketamine/rompun anesthesia (100mg/10mg) determined for each animal. The animals were fixed in the stereotaxic apparatus for surgical application. The skin over the head was dissected carefully with surgical blade and the skull was exposed. Dorsoventral position of the head was checked by relative coordinates of bregma and lambda and the head was set to be level with manipulation of the nose bar. Coordinates of bregma were recorded and the coordinates of either electrode or fiber placement according to the group were calculated. A small round hole was drilled carefully not to damage the underlying cortex. MS/VDB coordinates relative to bregma were +1.2 mm anteroposterior (AP), 0.0 mm mediolateral (ML), and -6.2 mm dorsoventral (DV) from dura for both MS/VDB-lesioned and sham-operated animals. An anodal current of 1.5 mA was applied for 20 s in electrolytically lesioned group, while a 2W 2s 980-nm diode laser application was made in the laser-lesioned group. Sham operations were identical to lesion operations except for current or laser application. Afterwards, the electrode or fiber was taken out and the skin was

carefully sutured. After surgery, a total of 3ml saline was injected subcutaneously into 3 sites.

3.3 Behavioral Testing

Behavioral testing was conducted between 1000 and 1500 hr for all tasks. Before testing each animal was individually kept for 10 min in the experiment room for habituation to the test environment.

3.3.1 Forced Swim Test

After 5 weeks of recovery, animals were tested in two forced swim tests (FST) separated by 24 h. Swim tests were conducted in a vertical Plexiglas cylinder with 45 cm height and 30 cm diameter that was filled with water (25 °C) to a height of 20 cm. In both tests animals were immersed individually into the water and allowed to swim; on the first day (FST1) the test duration was 15 min and in the second day (FST2) 5 min. The dependent variable was the total duration of immobility as measured for the first 5min of FST1 and entire 5 min of the FST2. Immobility was defined as floating or lack of motion of the entire body without leaning against the wall of the cylinder.

3.3.2 Morris Water Maze

One week after the second forced swim test, animals were tested on a spatial navigation test using a MWM tank with a hidden platform that was surrounded by a large number of objects providing distinct cues. The tank was a circular Plexiglas pool (120 cm diameter, 60 cm height) filled to a height of 40 cm with water of 20.0±2.0 °C temperature. A transparent platform (10 cm x 10 cm) was placed in a fixed quadrant throughout the training session submerged in water with the top 2 cm below water level. Animals received 7 days of training with 5 trials on each day with a 10 min intertrial interval. On each trial, the animal was randomly immersed into water from one of four designated starting points

and was allowed to swim for 60 s or until it climbed onto the platform. The duration of this swimming interval was recorded as escape latency. Animals stayed on the platform for 15 s before being taken out. When an animal failed to reach and climb onto the platform within 60 s, it was gently guided by hand to the platform and allowed to stay there for 15 s. One day after the 7-day training period, animals received a single probe trial where each subject entered the pool from a fixed point and was allowed to swim for 2 minutes in the absence of the platform. The total time spent in the quadrant where the platform was placed in the acquisition phase of the experiment was recorded. One day after the probe trial, animals were subjected to 5 trials with a visible platform in order to ensure that the animals did not have any sensory-motor problems. In this session, a flagged platform was placed into the pool such that it was be 2 cm above the surface of the water. The location of the platform was changed in each trial, while the point that the animals were immersed into water was set constant throughout the session.

3.3.3 Open Field Test

Two weeks after the MWM task, animals were administered the open field test. The open field (OF) apparatus was a square box (80cm x 80cm x 40cm) with the floor of the apparatus divided into 64 squares. Each subject was allowed to explore the field for 5 minutes and the number of squares crossed with both fore- and hind legs served as a measure of locomotor activity.

3.4 Histology

At the end of behavioral testing, animals were sacrificed with an overdose of ketamine and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde (PFA) with 20% glutaraldehyde in phosphate buffer. Brains were removed and fixed with the paraformaldehyde solution for one week. Vibratome sections of 50 micron thickness were taken. Every section was mounted onto gelatin-coated slides. Half of the sections were stained with Cresyl Violet and the other half was used for histological verification of AChE content.

4. RESULTS

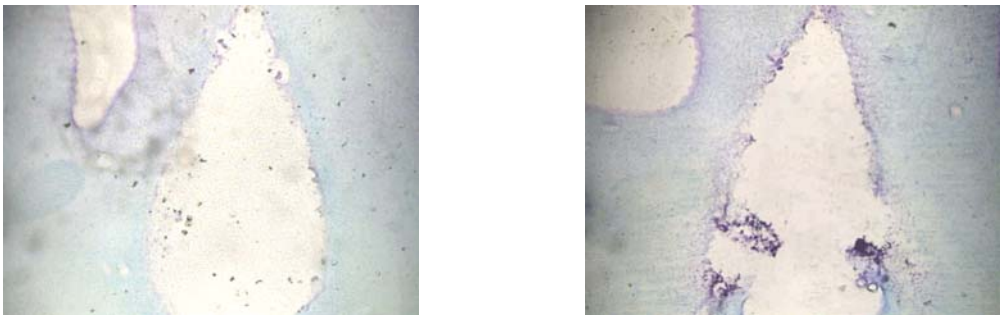
4.1 Histology

According to a preliminary histological inspection, two of the animals from the electrolytical lesioned group were excluded from further examination since one had a tumor-like structure developed in the cortex, while the other had a unilateral lesion. Additionally two animals from the laser-lesioned group were excluded because of extensively large size and posterior location to MS/VDB region in one and lateral location in the other. The remaining 24 lesioned (electrolytic n=12; laser n=12) and all sham operated animals (electrode n=8; laser fiber n=8) were used for further histological and behavioral examination.

Results from histological examination of cresyl violet and AChE stained sections revealed no sign of a brain tissue damage in either electrode or fiber sham-operated animals (group name: sham). On the other hand, among the lesioned animals the presence of three different variables has been observed under light microscope with respect to the ablated area; these are the location of the lesion (anterior-posterior), the size of the lesion (large-small), and the specific reduction of AChE in the hippocampal formation (Low-AChE and High-AChE). High-AChE and sham groups were identified to have high contrast among subdivisions of the hippocampus, while in Low-AChE animals dense staining was not present specifically in the hippocampus but preserved in other structures (Figure 4.3). All of these variables were assigned according to observations under light microscope.

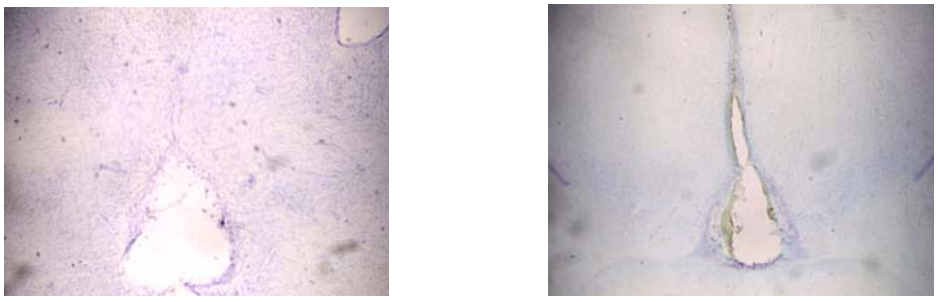
Each lesioned animal (electrolytic and laser groups) was assigned to a group according to the aforementioned factors. Overall classification of lesions reveal 9 animals (electrolytic n=7; laser n=2) with low AChE content (group name: Low-AChE) and 15 animals (electrolytic n=5; laser n=10) with high AChE (group name: High-AChE); 11 animals had large lesions (electrolytic n=6; laser n=5) (group name: large), 13 animals had small lesions (electrolytic n=6; laser n=7) (group name: small); 15 animals displayed anterior lesions (electrolytic n=7; laser n=8) (group name: anterior) and 9 animals had

posterior lesions (electrolytic n=5; laser n=4) (group name: posterior). The numbers of animals in each group are summarized in Table 4.1. Pictures of representative laser and electrolytic lesions are depicted in Figure 4.1 and Figure 4.2.



(a) (b)

Figure 4.1 Representative pictures of large MS/VDB area lesions created with (a) 980-nm diode laser and (b) electrocoagulator (Cresyl violet staining, X 4).



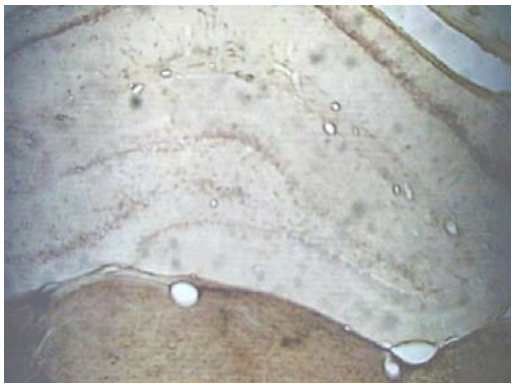
(a) (b)

Figure 4.2 Representative pictures of small MS/VDB area lesions created with (a) 980-nm diode laser and (b) electrocoagulator (Cresyl violet staining, X 4).

Table 4.1
The number of lesioned animals in groups categorized by the type of the lesion

	Laser lesion	Electrolytic lesion
Low-AChE	n = 2	n = 7
High-AChE	n = 10	n = 5
Anterior lesion	n = 8	n = 7
Posterior lesion	n = 4	n = 5
Large lesion	n = 6	n = 5
Small lesion	n = 6	n = 7

Hippocampal AChE content have been classified to be “Low-AChE” or “High-AChE” and served to categorize the lesioned animals functionally (Figure 4.3). Thus, the main discrimination was made accordingly and the number of animals with different lesion location and size are illustrated in Table 4.2 in terms of their AChE content in the hippocampus. Apparently large, anterior, dorsal lesions of MS/VDB region lead to decrease in hippocampal AChE content whereas high AChE content in hippocampus is retained in some animals despite lesions.



(a)

(b)

(c)

Figure 4.3 Representative pictures of hippocampus from (a) Low-AChE, (b) High-AChE and (c) sham-operated control animals (AChE staining, X 4).

Table 4.2
The number of lesioned animals according to AChE content in the hippocampus and lesion size and location.

	Low AChE	High AChE
Large lesion	n = 7	n = 4
Small lesion	n = 2	n = 11
Anterior lesion	n = 8	n = 7
Posterior lesion	n = 1	n = 8

4.2 Forced Swim Test

Total durations of immobility (mean and standard deviation) in the first 5 min of forced swim test 1 (FST 1) and entire 5 min of forced swim test 2 (FST2) are presented in Table 4.3.

Table 4.3

Mean and standard deviation of the duration of immobility (sec) during FST1 and FST2 for lesioned and sham-operated groups.

Group		Immobility (sec)	
		FST1	FST2
Low AChE (n = 9)	Mean	61.11	75.78
	SD	22.12	28.90
High AChE (n = 16)	Mean	50.33	54.27
	SD	22.02	24.09
Sham (n = 16	Mean	50.75	47.44
	SD	20.55	26.64

The immobility scores of Low-AChE, High-AChE and sham-operated controls in FST1 and FST2 are represented in Figure 4.4.

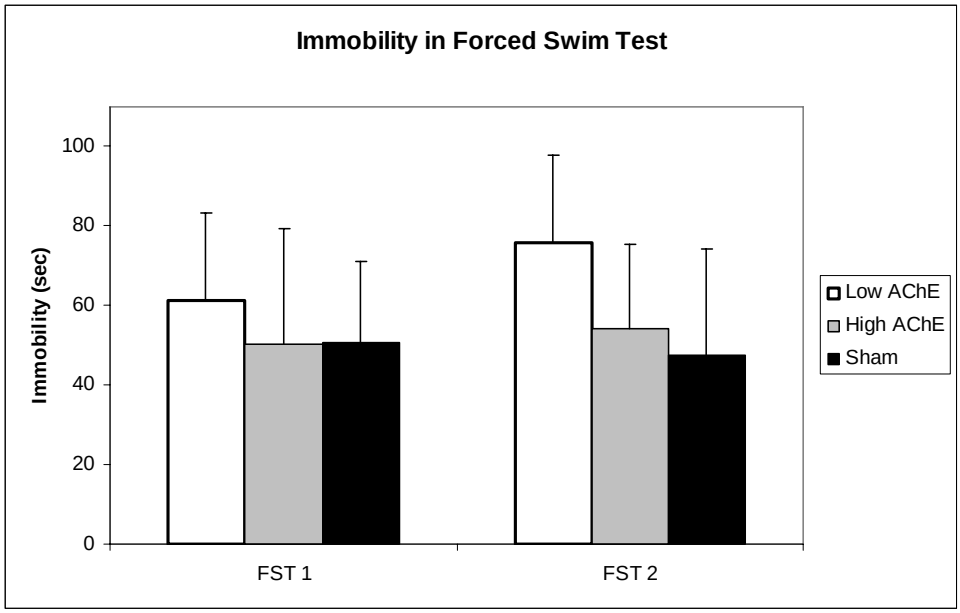


Figure 4.4 Duration of immobility (sec) in FST1 and FST2 for Low-AChE, High-AChE and sham animals. (Duration for the Low-AChE is significantly higher in FST2 than for the other two groups).

A one way analysis of variance (ANOVA) revealed that immobility durations in the second swim test differ significantly from each other as a function of hippocampal AChE content [$F(2, 39) = 4.07$, $p < .05$]. Least significance difference (LSD) post-hoc tests revealed that Low-AChE animals displayed significantly longer immobility duration compared to High-AChE and sham-operated controls. Moreover the size of the lesion also has significant impact on immobility in FST2 [$F(2, 39) = 4.41$, $p < .05$] and LSD post-hoc test revealed large lesions to aggravate the immobility compared to sham-operated control group. However, that effect appears to be related to AChE content [$F(2, 28) = 3.69$, $p < .05$] since LSD post-hoc test revealed that only subjects with large lesions and Low-AChE differ from sham-operated controls in the immobility scores of FST2 but not the ones with large lesions and High-AChE. Neither the anterior-posterior elongation [$F(2, 39) = 1.95$, $p > .05$] nor the type of the lesion [$F(2, 39) = 1.80$, $p > .05$] appeared to affect significantly the duration of immobility in FST2. No significant differences were found in immobility scores of FST1 according to AChE content [$F(2, 39) = 0.32$, $p > .05$], type of the lesion [$F(2, 39) = 0.80$, $p > .05$], size of the lesion [$F(2, 39) = 0.17$, $p > .05$] or location of the lesion [$F(2, 39) = 0.24$, $p > .05$].

4.3 Morris Water Maze

Performance of Low-AChE, High-AChE and sham-operated groups for 7 days of the acquisition trial, visible-platform trial and probe trial MWM are summarized in Table 4.

Table 4.4
Mean and SD of Escape Latencies (sec) during 7 days of acquisition period, one-day visible platform and probe trial MWM for Low-AChE, High-AChE and sham-operated groups.

	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Visible Platform	Probe Trial		
									First min	Second min	Total
Low AChE (n=9)											
mean	40.09	32.62	20.93	19.27	12.56	8.24	6.84	11.49	23.89	23.33	42.22
SD	14.97	13.51	10.84	9.61	8.12	3.63	3.86	3.27	6.83	5.70	6.76
High AChE (n=15)											
mean	39.61	17.76	12.84	12.40	9.12	7.83	6.12	14.15	25.53	17.13	42.20
SD	9.15	8.59	7.61	5.25	4.96	4.17	2.65	10.08	5.88	3.93	6.78
Sham (n=16)											
Mean	43.00	27.83	12.69	13.33	11.34	9.78	7.51	12.08	26.38	20.06	46.44
SD	12.76	15.05	8.28	6.57	7.50	5.02	5.88	6.31	4.19	4.54	6.58

The acquisition performance of Low-AChE, High-AChE and sham-operated controls in the 7 day MWM training is represented in Figure 4.5.

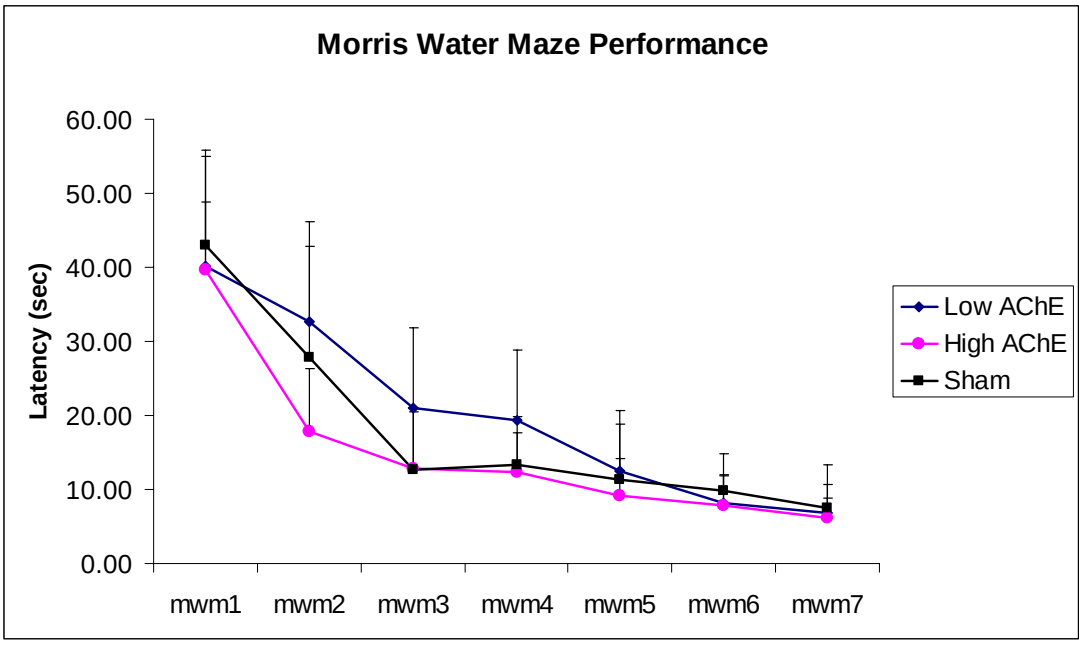


Figure 4.5 The acquisition performance of Low-AChE, High-AChE and sham-operated controls during the 7 day MWM training.

A one way ANOVA revealed main effect of hippocampal AChE content on latency scores to find the hidden platform in MWM training in the second [$F(2, 39) = 4.33$, $p < .05$], third [$F(2, 39) = 4.11$, $p < .05$] and fourth [$F(2, 39) = 3.35$, $p < .05$] days. LSD post-hoc tests revealed that Low-AChE animals displayed significantly longer latency compared to High-AChE animals in the second day while on the third and fourth day they differed significantly from both high-AChE and sham-operated controls. No difference was found in the first day [F (2, 39) = 0.24, $p < .05$], fifth day [F (2, 39) = 1.03, $p < .05$], sixth [F (2, 39) = 0.41, $p < .05$] and seventh days [F (2, 39) = 0.26, $p < .05$] as a function of AChE. No significant effect was found according to the type, size and location of the lesion. A repeated measures ANOVA revealed significant within-subjects effect for days of testing in MWM [$F(6, 186) = 42.24$, $p < .001$] while lesion type [$F(6, 186) = 0.22$, $p > .05$], lesion size [$F(12, 186) = 0.82$, $p > .05$], lesion location [$F(6, 186) = 0.71$, $p > .05$] as well as hippocampal AChE content [$F(6, 186) = 1.23$, $p > .05$] had no effect.

The latency to find visible platform did not reveal any significant effect of neither of the parameters (Figure 4.6).

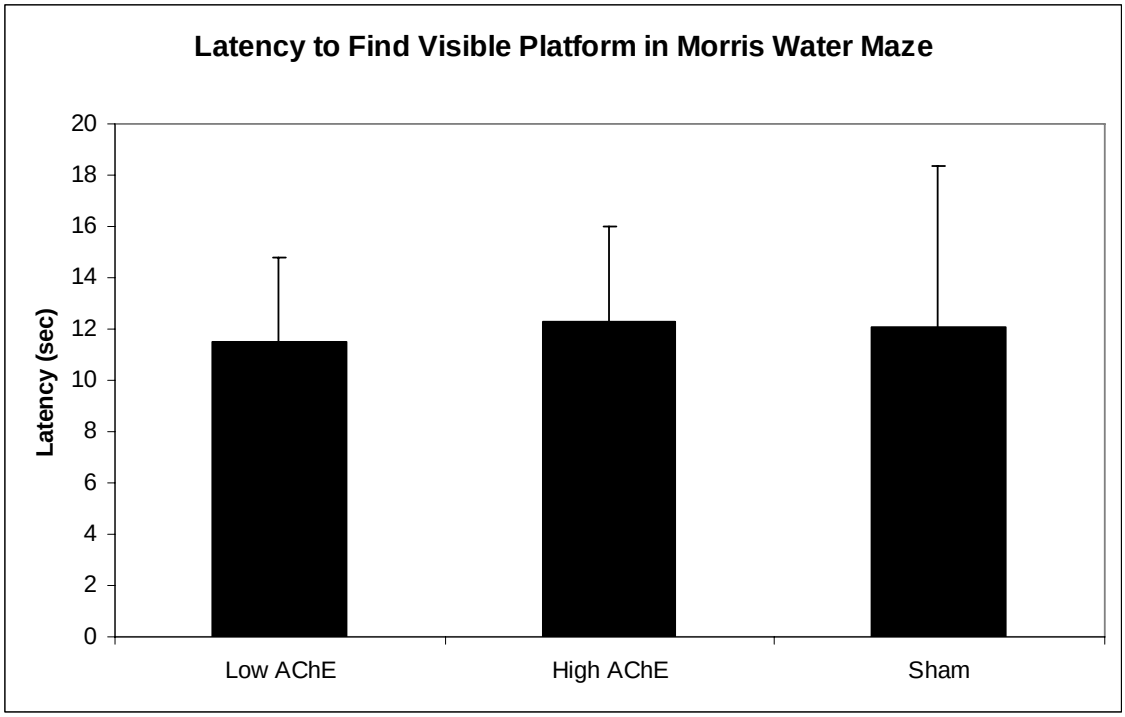


Figure 4.6 Mean and standard deviation of escape latencies (sec) of the Low-AChE, High-AChE and sham animals in the MWM when the platform is visible.

Time spent in the target quadrant in the first and second minutes of MWM probe trial is illustrated in Figure 4.7.

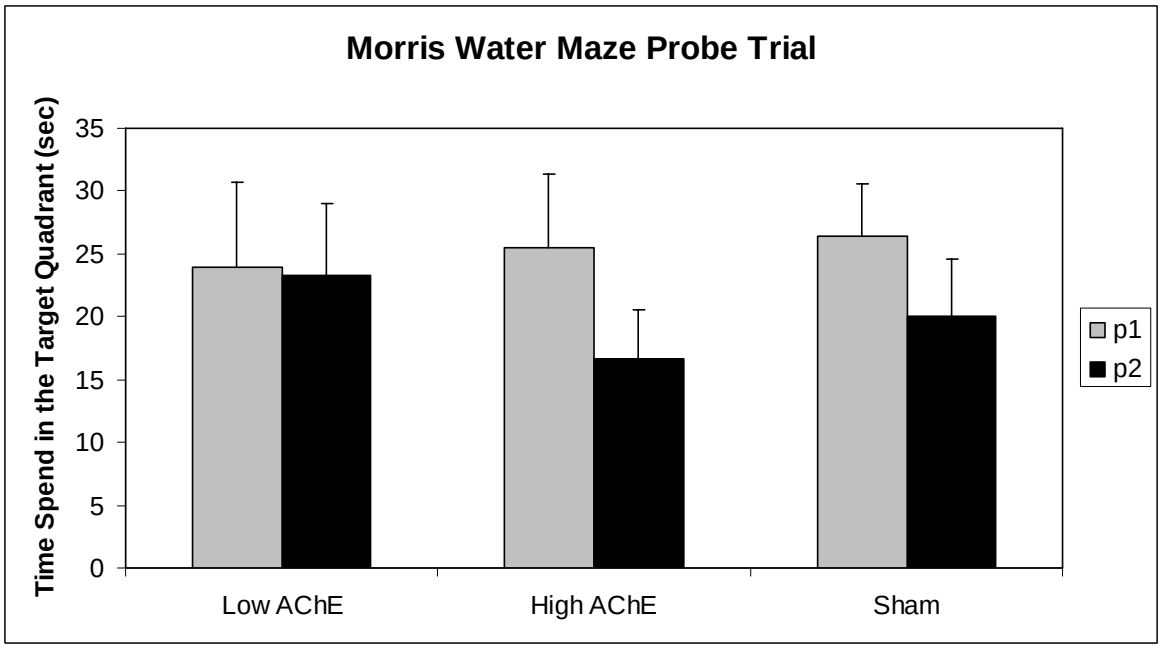


Figure 4.7 Mean and Standard deviation of time (sec) spent in the target quadrant during the first (p1) and second (p2) minutes of MWM probe trial.

A one way ANOVA showed main effect in the second minute of the probe trial as a function of hippocampal AChE content [F (2, 39) = 3.64, p < .05]. LSD post-hoc tests revealed a significant difference between Low-AChE and High-AChE. No significant effect was detected in the total swim duration in the target quadrant (where the platform was located during the acquisition trials) according to the type [F (2, 39) = 1.52, p > .05], size [F (2, 39) = 3.15, p > .05], location [F (2, 39) = 0.51, p > .05] or AChE content [F (2, 39) = 0.55, p > .05].

4.4 Open Field

Squares crossed (Mean and Standard deviation) in open field test of Low-AChE, High-AChE and sham-operated groups are summarized in Table 5 and illustrated in Figure 4.8.

Table 4.5
Mean and SD of number of squares crossed during open field test.

Group		No of Squares
Low-AChE (n = 9)	Mean	37.89
	SD	37.96
High-AChE (n = 16)	Mean	41.64
	SD	25.24
Sham (n = 16	Mean	45.31
	SD	27.07

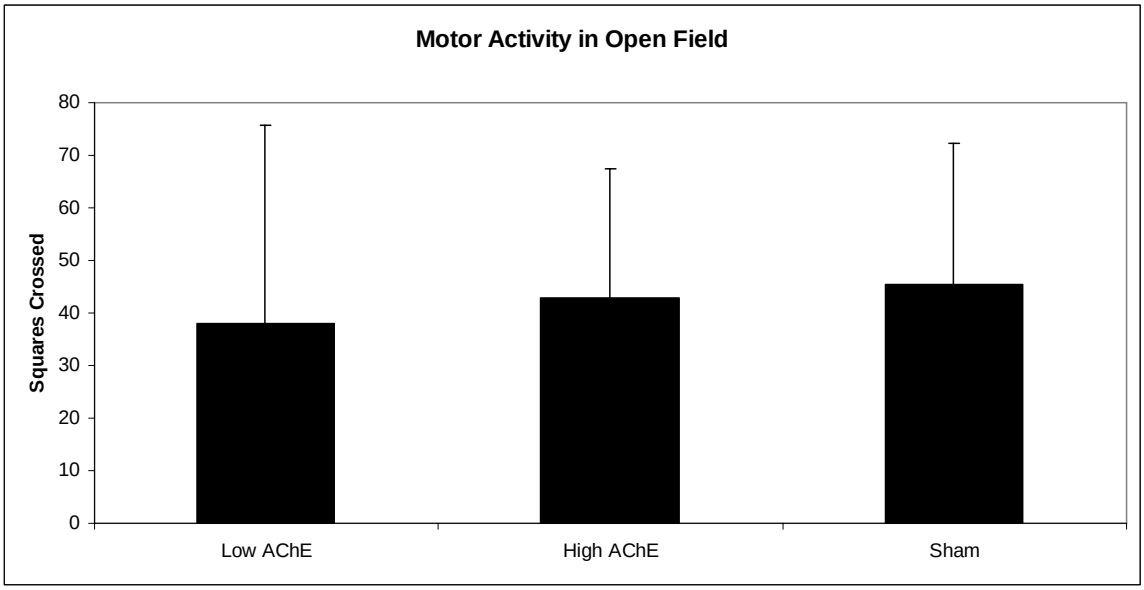


Figure 4.8 Mean and Standard deviation of the number of squares that animals crossed in the open field test

The one way ANOVA revealed that the number of squares crossed did not differ significantly between the groups according to hippocampal AChE content [F (2, 39) = 0.44, $p > .05$], type [F (2, 39) = 0.37, $p > .05$], size [F (2, 39) = 0.15, $p > .05$] or location [F (2, 39) = 0.52, $p > .05$] of the lesion.

5. DISCUSSION

The present study aimed to investigate the consequences of hippocampal denervation in terms of irreversible MS/VDB nuclei lesioning on behavioral despair and navigational learning. To that purpose MS/VDB lesions were achieved electrolytically or with 980-nm diode laser beam application in rats brain. The functional outcome of the lesions appeared as a reduction in AChE content of the hippocampus in some of the lesioned animals compared to sham-operated control animals. Behavioral investigation revealed that the elimination of cholinergic supply of hippocampus via destruction of the medial septum aggravates behavioral despair in rats. Specifically, duration of immobility was significantly augmented in the second but not in the first of two swim tests in rats with decreased hippocampal AChE compared to those with normal AChE content. Additionally laser or electrolytic lesions do not seem to impair performance in the Morris water maze task, often used to assess navigational learning [112]. In that respect the present study reveals an additional insight about the co-morbidity of depressive states and impaired learning. The behavioral findings in the present study appear not to be due to possible impairment of the sensory-motor capabilities of the lesioned animals since they performed as well as sham controls in the visible platform version of MWM and open field activity test. Current results reveal differences in the success of MS/VDB lesions created by laser and electrocoagulator device. Electrolytic lesions appear to be more reliable compared to laser lesions in terms of the reduction of AChE content in hippocampus.

5.1 Histochemical Effects of MS/VDB Lesions

The functional effects of MS/VDB lesions are thought to be exerted primarily on the hippocampal formation keeping in mind the dense connections between these two structures [24-26, 40]. This and several other studies have consistently revealed that complete electrolytic, neurotoxic or immunotoxic lesions of the medial septal area result in reduction of the hippocampal AChE content most probably due to decreased ACh delivery to the hippocampus. On the other hand, incomplete lesions have been shown to spare

cholinergic activity subsequent to destruction [108]. Apparently, in the present study large, anterior, dorsal lesions of MS/VDB region caused decrease in hippocampal AChE content whereas high AChE content in the hippocampus was retained in some animals despite damage in the brain tissue. This phenomenon parallels results reported by Oderfeld et al. (1977) who have been shown that only total MS/VDB destruction is accompanied by extensive AChE depletion (about 90% decrease) in the hippocampus. In that respect hippocampal AChE content can be treated as a reliable marker of the behavioral success of MS/VDB lesions.

5.2 Electrolytic Versus 980-nm Diode Laser Lesions of MS/VDB

In the present study, electrolytic lesions were found to be more successful compared to laser lesions in terms of the decrease of AChE content in the hippocampus. Although the same coordinates were used for both types of lesion, electrolytic and laser lesions of the medial septal area were found to differ in size and location as well as functional outcome. Histological analysis of the three dimentional distribution of laser and electrolytic lesions revealed similar patterns as assessed by Tabakoglu in his thesis research [113]. Namely, electrolytic lesions have an ellipsoidal shape that elongates in the anterior-posterior direction whereas laser lesions are found to extend in the ventral direction. The results are also in accordance with previous parametric research which showed that a 2W-2s laser application creates an ablation area comparable to electrolytic lesions by 1.5 mA current application for 20s [10, 11]. Possible differences in the lesion parameters in the present study might have occurred due to the temporal immune response in the surrounding area of ablations [16, 17]. In that respect behavioral testing was started approximately 40 days post-lesion that is assumed to be an adequate delay to avoid the differential immune response between electrolytic and laser lesions [20-23]. Altogether previous research was limited to days and weeks of post-operative duration and does not reveal the possible consequences in terms of months. Moreover, the use of different brain areas among studies might also be a reason for differences in histological and functional results following different lesioning techniques. Overall histological examination points to the success of electrolytic lesions over 980-nm diode laser lesions particularly in the medial septal area

and to the need for further research on parametric optimization that is currently beyond the scope of the present study.

5.3 The Effects of MS/VDB Lesions On Behavioral Despair

To the authors knowledge, this is the first report in the literature to show that MS/VDB lesions aggravate behavioral despair in rats compared to sham-operated controls as assessed by an increased duration of immobility in the second forced swim test. Moreover, that effect appears to be related specifically to the decrease in hippocampal AChE content since animals with relatively high content of AChE in their hippocampus displayed similar behavior to sham-operated controls. Immobility in the second forced swim test has been shown to be shortened by major classes of antidepressants as well as phototherapy like light exposure [50, 51]. Further evidence comes from the studies that involve the analysis of plasma corticosteroid levels found to be elevated due to HPA axis dysregulation in major depression of humans [49] and after forced swim test in animals [57-59]. Thus, it appears noteworthy to suggest that the cholinergic denervation of hippocampus reflects augmentation in a depression like behavioral deficit as has been shown by longer immobility durations in the second forced swim test. Since there was no significant effect of lesion or AChE content on the first swim test, it is possible that medial septal lesions interfere with the induction of behavioral despair that becomes evident in the second swim test. The current finding makes greater sense when the inhibitory feedback role of the hippocampal formation on HPA axis is considered [62]. This effect is thought to be regulated by the densely expressed glucocorticoid and mineralocoticoid receptors in hippocampal formation as well as medial septal area [66-69]. That mechanism has been studied by Gesing et al. (2001) who showed increases in hippocampal MR mRNA after 8 and 24h delay after a single forced swim test. Furthermore, the level of acetylcholine and/or AChE in the hippocampus also appears to contribute to the severity of depressive states [73, 74]. The hippocampus, although one of the most extensively studied regions in the brain, is actually a difficult structure for direct manipulations due to its large volume, distinct subdivisions and important role in variety of cognitive functions [114]. The current study indicates that indirect manipulation of the hippocampal formation via destruction of

its afferent connections from medial septal area might lead to an impairment of hippocampal regulation on affective states. Acetylcholine being the major neurotransmitter in the septohippocampal complex appears to be the primary candidate in that phenomenon. Supporting evidence comes from the fact that Low-AChE animals are found to differ significantly from high-AChE animals as well as sham-operated ones. However, with the nonspecific lesioning method applied in the current study it is not possible to distinguish certainly which of the neurotransmitter systems (ACh, GABA, and Glu) is responsible for the resulting behavioral alterations. Also the possibility of the involvement of more than one system remains to be discovered. Additionally, specific contribution of the medial septal area to depression should be addressed by sparing the passing fibers such as is possible with intraseptal administration of neurotoxic substances.

5.4 The Effects of MS/VDB Lesions On Navigational Learning

The present results indicate that animals are capable of learning the MWM task and eventually achieve short latencies (<10 sec in the last two days) to find the submerged platform regardless of the type and size of the lesions or hippocampal AChE content. However, day by day analysis of variance revealed that animals with Low-AChE content in the hippocampus performed worse than High-AChE and sham operated control animals in the second, third and fourth day of MWM acquisition trials. In that respect Low-AChE animals appear to have difficulty in acquiring the demands of the task that is resolved with additional training.

Much effort has been dedicated to investigate the role of the medial septal area on cognitive tasks. Several studies reported severe learning and memory impairments in spatial tasks after non-specific septal lesions as well as hippocampal denervation by fimbria-fornix removal [87-89]. On the other hand, evidence should be considered in the light of conflicting results that report the involvement of medial septal area in some learning paradigms (such as spatial long-term memory) but not others (such as contextual learning) [109, 114]. Current approach to clarify this discrepancy is directed towards the elucidation of the potential roles of different neurotransmitter systems such as ACh and GABA in learning tasks [28, 111]. Development of immunotoxic drugs has allowed for

more reliable methodology in that manner; however, controversy remains since some of the studies report learning impairments following either cholinergic or GABAergic destruction [107, 108] whereas others do not [110]. The overall picture reveals that the learning and memory impairments caused by medial septal area dysfunction depend on the demands of the learning task and training procedure as well as the type and location of the brain damage.

In addition to the hypothesis of cognitive decline caused by cholinergic deficiency, learning impairments of the animals with denervated hippocampus have also been attributed to deficiency in attention and/or motivation [115] as well as lack of ability to choose the best strategy rather than total learning incapability [104, 106, 107, 110].

The present results do not show memory impairment for location of the platform in the probe trial of the MWM task. However, a minute by minute analysis of the two minute probe trial revealed that animals with Low-AChE content differed from High- AChE and sham-operated control animals in terms of their temporal searching strategy. Namely, Low- AChE animals did not decrease the time spent in the target quadrant to search the rest of the maze like High-AChE and sham-operated control animals. Gray and McNaughton (1983) have proposed that hippocampal activity is important in suppression of conflicting or competing information such that in the case of frustration goal seeking is avoided [114-119]. The stimulatory role of medial septum in spontaneous alternation also suggests that the animals with damage to the medial septal area would have less drive toward exploration of unknown maze areas [120].

5.5 The Effects of MS/VDB Lesions On Sensory-motor Functions

No difference among the groups was detected in the visible platform trials of MWM or motor activity as assessed by open field exploration. Thus, significant results in the behavioral despair as well as performance in the probe trial of MWM appear to be independent of motor capabilities but is attributable to specific affective as well as cognitive states.

5.6 The Effects of MS/VDB Lesions On The Association of Depression and Impaired Learning

Research with humans and animals indicate a high degree of co-morbidity of depression and impairment of learning and memory. Induced depression usually results in impaired learning and memory as well. A recent study by Naudon and Jay (2005) revealed that animals which display high-immobility compared to animals with low-immobility in FST perform worse in a spatial learning task RAM, but do not differ in a non-spatial object recognition task. Similarly an unpublished data from our laboratory reveals that female rats with higher immobility in the second of two forced swim tests are worse learners in MWM (Canbeyli, unpublished study). Not only forced swimming but also other animal models of depression such as open space swim [122], learned helplessness or chronic mild stress [123] as well as olfactory bulbectomy [124] contributes to impaired spatial learning.

Keeping in mind the profound role of the hippocampus on stress response as well as learning and memory paradigms, medial septal area that has extensive connections with hippocampal formation has been thought to be a good candidate in the continuum of seeking the brain regions involved in the co-morbidity of depressive states and impaired learning. A preliminary study conducted in female rats has shown that medial septum lesioned subjects display aggravated behavioral despair in forced swim test and impaired learning ability in MWM (Canbeyli, article in preparation). However, the present results fail to imitate the findings with female rats in terms of impaired navigational learning. Rather, damage to the medial septal area that causes decrease in hippocampal AChE aggravates depression like behavior without significantly impairing spatial navigation in male rats.

Although many studies point to the existence of association between depressive states and impaired learning, others report dissociation between depression and learning. A recent study by Pezuk et al. (2006) showed that BNST, a limbic structure that aggravates behavioral despair, fails to cause impaired navigational learning in a MWM task in both female and male subjects [125]. Additionally, Flinders Sensitive Line (FSL) rats in spite of their behavioral resemblance to depressed humans [126, 127] do not display spatial learning impairment [128]. It is important to note that these rats exhibit higher sensitivity

to cholinergic agonists via elevated mACh receptor numbers in the hippocampus [80, 81]. Thus, hippocampal cholinergic system itself might have a limited effect on the association of depression and learning impairments. The present results, therefore suggest that low acetylcholine level in the hippocampus, marked by low AChE content, might be sufficient to aggravate depression but fail to impair navigational learning.

6. CONCLUSION AND FUTURE WORKS

The present study reveals that lesioning the medial septal area aggravates behavioral despair in rats. That effect was found to be specific for the reduced AChE content in the hippocampus. On the other hand, despite the fact that MS/VDB damage does not contribute to impaired learning abilities compared to sham-operated ones, lowered AChE content in the hippocampus affects the performance in the acquisition trials of first four days. Thus, low AChE content in the hippocampus appears to be sufficient to predict aggravated depression but not impairment in at least one type of learning, namely spatial navigation.

The lesioning methodology used in the present study does not discriminate whether MS/VDB nuclei or the fibers passing through that area are responsible for the outlined behavioral outcomes. To clarify that issue, it would be necessary to utilize neurotoxic compounds that target neuronal cell bodies in the medial septal area. Additionally, the presence of different neurotransmitter systems in the medial septal area suggests the need of further research to identify in detail the role of septohippocampal complex in depression and navigational learning.

Electrolytic lesion of MS/VDB results in higher number of Low-AChE subjects compared to 980-nm diode laser lesions. That particular outcome might be related to the three dimensional distribution of the ablated area. Electrolytic lesions appear to elongate in anterior posterior direction while laser lesions are more likely to extend ventrally. Since MS/VDB occupies a brain region that is lengthened in anterior-posterior rather than dorso-ventral direction, it might be suggested that the electrolytically created lesions favor complete MS/VDB damage in comparison to 980-nm diode laser application.

To be able to obtain comparable functional effects following MS/VDB lesioning, namely low AChE content in hippocampus, further research should be addressed to optimize the coordinates for 980-nm diode laser lesions of MS/VDB area.

APPENDIX A. EQUIPMENT FOR NEUROSURGERY

Stereotaxic Instrument: Stoelting. (USA)

980-nm Diode Laser: Opto-Power OPC-D010-980-FCPS (Max. Power: 10 Watt)

Electrocoagulator: Teknofil Inc. (Turkey)

400-μm silica optical fiber: Spindler-Hoyer

Driller: Proxxon Minimot-40 (Check Republic)

Surgical silk with needle: Doğsan (Turkey)

Forcepts: Hartmann (Germany)

Tissue Forcepts: Hartmann (Germany)

Micro Dissecting Scissors: Hartmann (Germany)

Bone Cutting Forcepts: Hartmann (Germany)

Scalpel Blades-number 2: Wuxi Xinda Medical Device Co., Ltd.

Scalpel Handles: Hartmann (Germany)

Needle Holders: Hartmann (Germany)

Spatula (Freer): Hartmann (Germany)

APPENDIX B. BUFFERS AND SOLUTIONS

B.1 Buffers and Chemicals For Perfusion

B.1.1 Preparation of 1L 4% Paraformaldehyde (PFA) – glutaraldehyde in 0.1 M PB

B.1.1.1 Materials

- Paraformaldehyde (PFA)
- 0.5M sodium hidroxyde (NaOH)
- 0.2M phosphate buffer (PB)
- 25% glutaraldehyde
- distilled H_2O

B.1.1.2 Method

- 40 gram PFA was added to 400 ml of distilled water
- This solution was dissolved by heating less than 60°C.
- 0.5 M NaOH was added dropwise until whitening dissappears
- 500ml 0.2M PB was added to that solution and dissolved
- 20 ml 25% glutaraldehyde was added and the final volume is completed to 1L with distilled H_2O

B.2 Buffers and Chemicals for Histology and Histochemistry

B.2.1 Preparation of 1 L 0.2 M Phosphate Buffer (PB)

B.2.1.1 Materials

- monobasic sodium phosphate (NaH_2PO_4)
- bibasic sodium phosphate (Na_2HPO_4)
- distilled H_2O

B.2.1.2 Method

- **Solution A**: 6.9 g monobasic sodium phosphate (NaH_2PO_4) dissolved in 250 ml of distilled H_2O.
- **Solution B**: 14.2 g bibasic sodium phosphate (Na_2HPO_4) dissolved in 500 ml of distilled H_2O.
- 115 ml Solution A was mixed with 385 ml of Solution B. pH range of final solution should be between 7.2 and 7.4.

B.2.2 Chrome-gelatinized Slides

B.2.2.1 Materials

- Gelatine
- Chromic Potassium Sulphate
- 70% ethanol
- Distilled H_2O
- Slides

B.2.2.2 Method

- 2.5 gr gelatine was dissolved in 500ml dH_2O by heating less than 60°C.
- 0.25 gr chromic potassium sulphate was added when gelatine is dissolved.
- Slides were rinsed in 70% ethanol followed by dH_2O (5min each)
- Slides were dipped in the chrome-gelatine solution twice and left over night at 37°C to dry

B.2.3 Cresyl Fast Violet (CFV) Staining

B.2.3.1 Materials

- Cresyl Fast Violet
- Acetic Acid ($C_2H_4O_2$)
- 70% ethanol
- 80% ethanol
- 100% ethanol

- Xylene
- Entellan
- Distilled H_2O
- Chrome-gelatinized slides
- Coverslips

B.2.3.2 Method

- 1 gram of Cresyl Fast Violet Stain was dissolved in 100 ml of dH_2O
- 0.25 ml Acetic Acid ($C_2H_4O_2$) was added to this solution and stirred for at least 24hr before use.
- 50 μm thick coronal brain slices were placed on chrome-gelatinized slides and let to dry at room temperature
- Slides were immersed in CFV stain for 10min
- Slides were passed alcohol gradient given below.
  i) 70% Alcohol (5 minutes)
  ii) 80% Alcohol (5 minutes)
  iii) 100% Alcohol (until desired discolorization of slides is achieved)
- Slides were dipped in Xylene followed by entellan application and closed with coverslip

B.2.4 AChE Histochemistry

B.2.4.1 Materials

- Acetylthiocholine iodide
- 0.1 M Maleic acid (pH 6.0)
- 10M sodium hidroxyde (NaOH)
- 50mM Tris solution (pH7.4)
- Hydrochloric acid (HCl)
- 5 mM Potassium ferricyanide
- 40mM Copper sulphate
- 100mM Sodium citrate
- DAB kit
- Xylene
- Entellan
- Distilled H_2O
- Chrome-gelatinized slides
- Coverslips

B.2.4.2 Preparation of Buffers and Solutions

- Maleic buffer (MB) was prepared by adjusting the pH of 0.1 M maleic acid to 6.0 with 10M NaOH
- Tris buffer (TB) was prepared by adjusting the pH of 50 mM tris solution to 7.4 with HCl
- Solution A was prepared in following order:
  1. 6.5 ml MB
  2. 0.5 ml sodium citrate
  3. 1.0 ml copper sulpate
  4. 1.0 ml dH_2O
  5. 1.0 ml potassium ferricyanide
- Solution B

  6 mg acetylthiocholine iodide was dissolved in 9ml MB

B.2.4.3 Method

- Brain slices were rinsed in MB for 5min
- Slices were pre-incubated in 1:100 dilution of Solution A in MB at room temperature for 30 min
- Slices were incubated in 1:1:100 dilution of Solution A and Solution B in MB at room temperature for 30 min
- Slices were rinsed in TB twice (1 min each)
- Slices were colored with DAB kit
    1. 6.5 ml MB
    2. 0.5 ml sodium citrate
    3. 1.0 ml copper sulpate
    4. 1.0 ml dH_2O
    5. 1.0 ml potassium ferricyanide
- Slices were taken into dH_2O and placed on chrome-gelatinized slides and let to dry at room temperature.
- Slides were dipped in Xylene followed by entellan application and closed with coverslip

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