T.C.
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HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

THE ROLE AND IMPORTANCE OF DORSAL AND VENTRAL HIPPOCAMPAL CATECHOLAMINERGIC INNERVATIONS IN SOCIAL MEMORY OF RATS

Ahmed Algali SEDAHMED MUSA

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MASTER OF SCIENCE THESIS

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Ahmed Algali SEDAHMED MUSA

Supervisor: Assoc. Prof. Banu Cahide TEL

This thesis study has been approved and accepted as a Master dissertation in "Pharmacology Program" by the assessment committee, whose members are

Listed below, on 15.08.2022

Chairman of the Committee: Prof. Bülent ELİBOL

Hacettepe University, Faculty of Medicine, Department of Neurology

Advisor of the Dissertation: Assoc. Prof. Banu Cahide TEL

Hacettepe University, Faculty of Pharmacy, Department of Pharmacology

Member: Assist. Prof. Elif ÇINAR

Zonguldak Bülent Ecevit University, Faculty of Pharmacy, Department of Pharmacology

This dissertation has been approved by the above committee in conformity to the related issues of Hacettepe University Graduate Education and Examination Regulation.

29 Ağustos 2022

(Signature)

Prof. Müge YEMİŞCİ-ÖZKAN

Director

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In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except cited references. It was produced by me in consultation with supervisor Assoc. Prof. Banu Cahide TEL and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences.

(Signature)

Ahmed Algali SEDAHMED MUSA

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ABSTRACT

Sedahmed Musa, A. A., The Role and Importance of Dorsal and Ventral Hippocampal Catecholaminergic Innervations in Social Memory of Rat, Hacettepe University Graduate School Health Sciences, Department of Pharmacology, Master of Science Thesis, Ankara, 2022. This study aims to investigate the role and importance of the catecholaminergic innervations in the dorsal (DH) and ventral hippocampus (VH) in social memory of rats. Female Wistar rats were injected bilaterally with 6-OHDA in either DH or VH. Then, following a 10-day recovery period, sets of behavioral tests were conducted to assess both motor and cognitive functions. In the open field locomotor activity test, the depletion of catecholamines in the VH increased the locomotor activity compared to naive, whereas in the DH it did not affect the locomotor activity. Regarding olfaction, there is no difference in the latency to find pellets between all groups in the buried food-seeking test. In addition, impairment in social memory has been observed as both the DH and VH groups were unable to recognize a familiar from a novel juvenile rat, while the VH group demonstrated a deficit in sociability in the 3-chamber social memory test. In the 2-trial direct interaction, the DH group failed to recognize a previously encountered juvenile rat confirming the previous test outcome. However, the VH group showed a reduction in time spent interacting with both familiar and novel juvenile rats, which indicates low sociability. Moreover, no difference was observed between the groups in the Y maze, novel object recognition, sucrose preference, or elevated plus maze tests. Using demonstrative immunofluorescence staining, a reduction of TH+ terminal has been observed in dorsal CA1, and ventral CA1 regions of the hippocampus in DH and VH groups respectively. This shows that, the catecholaminergic innervations in the CA1 region of the DH and VH are important in modulating rat social memory. Furthermore, the catecholaminergic innervation of the VH may be more significant than DH in controlling sociability and locomotor activity. Additionally, a minor role of this innervation in spatial, recognition, olfaction, and anxiety processing could be proposed, which cannot be demonstrated with such a mild lesion made in this study. These findings could highlight the importance of catecholaminergic innervations in the VH in the behavioral deficits associated with neurodevelopmental and neuropsychiatric disorders.

Keywords: Social memory, hippocampus, catecholamines, rat, 6-OHDA

ÖZET

Sedahmed Musa, A. A., Sıçanların Sosyal Hafızasında Dorsal ve Ventral Hipokampal Katekolaminerjik Innervasyonun Rolü ve Önemi, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Farmakoloji Programı Yüksek Lisans Tezi, Ankara, 2022. Bu tez çalışmasında, dorsal (DH) ve ventral hipokampüste (VH) bulunan katekolaminerjik terminallerin sosyal hafızadaki rolünün ve öneminin araştırılması hedeflenmiştir. Dişi Wistar sıçanlarında, bilateral DH'ye veya VH'ye 6-hidroksidopamin (6-OHDA) enjekte edilmiştir. 10 günlük iyileşme sürecinin ardından hem motor hem de bilişsel işlevleri değerlendirmek için davranış testleri yapılmıştır. Açık alan lokomotor aktivite testinde; VH enjeksiyon grubunda katekolaminlerin azalması sonucunda lokomotor aktivitede artış görülürken DH enjeksiyon grubunda değişiklik olmadığı gözlenmiştir. Koku alma ile ilgili gömülü yem arama testinde, tüm gruplar arasında yem bulmak için geçen sürede (yem bulma latansı) bir fark olmamıştır. Ayrıca, üç odacıklı sosyal bellek testinde, daha önce tanıdığı juvenil yeni sıçanı tanıyamadıkları için hem DH hem de VH gruplarında sosyal bellekte bozulma olduğu ve VH grubunda sosyallikte azalma olduğu gözlenmiştir. İki yönlü doğrudan etkileşim testinde, DH grubu, daha önceden tanıtılan yavru sıçanı tanıyamamıştır ve bu bulgu, üç odacıklı sosyal bellek testi sonucunu doğrulamıştır. Bununla birlikte VH grubu, düşük sosyalliği belirten hem tanıdık hem de yeni juvenil sıçanlarla etkileşime girmek için harcanan sürede azalma göstermiştir. Y labirent, yeni nesne tanıma, sükroz tercih ve yükseltilmiş artı labirent testlerinde gruplar arasında fark gözlenmemiştir. İmmünofloresan boyama yöntemi ile DH ve VH gruplarında sırasıyla hipokampüsün dorsal CA1 ve ventral CA1 bölgelerinde tirozin hidroksilaz+ (TH+) terminallerinde azalma gözlenmiştir. Bu sonuç, DH ve VH'nin CA1 bölgesindeki katekolaminerjik innervasyonnun, sıçan sosyal hafızasını modüle etmede önemli rol oynadığını göstermektedir. Ayrıca, sosyallik ve lokomotor aktiviteyi kontrol etmede, VH katekolaminerjik nöronları DH katekolaminerjik nöronlarından daha önemli rol oynayabilir. Ek olarak, bu nöronların uzamsal hafıza, tanıma, koku alma ve kaygı işlemede küçük bir rolü olduğu öne sürülebilir, fakat bu çalışmada hafif bir lezyon oluşturulduğu için net bir sonuca ulaşılamamıştır. Bu bulgular, nörogelişimsel ve nöropsikiyatrik bozukluklarla ilişkili davranışsal bozukluklarda VH'deki katekolaminerjik nöronların önemini vurgulayabilir.

Anahtar Kelimeler: Sosyal bellek, hipokampüs, katekolaminler, sıçan, 6-OHDA

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LIST OF ABBREVIATION

(Dbh-/-) : Dopamine beta-hydroxylase knockout mice

6-OHDA : 6-Hydroxydopamine

ADHD : Attention deficit hyperactivity disorder

ASD : Autism spectrum disorder

Avpr1b : Vasopressin 1b Receptor

BFT : Buried food seeking test

CA : Hippocampal cornu Ammonis

CA2 : Hippocampal cornu Ammonis subfield 2

CA3 : Hippocampal cornu Ammonis subfield 3

COMT : Catechol-O-methyltransferase

DA : Dopamine

dCA1 : Dorsal Cornu Ammonis subfield 2

DH : Dorsal hippocampus

DOPA : Dihydroxyphenylalanine

EPM : Elevated plus maze test

LC : Locus coeruleus

MAO : Monoamine oxidase

MOE : Main olfactory epithelium

NAc : Nucleus accumbens

NE : Norepinephrine

NGS : Normal goat serum

NMDA : N-methyl-D-aspartic acid

NOR : Novel object recognition test

O.F : Open filed

Pak2+ : Protein kinase 2

PBS : Phosphate-buffered saline

PBS-t : Phosphate-buffered saline with Tween 20

RN : Midbrain raphe nuclei

Sham-D : Animals injected with the sham in the dorsal hippocampus

Sham-V **:** Animals injected with the sham in the ventral hippocampus

Shank2 **:** SH3 And Multiple Ankyrin Repeat Domains 2

SMT1 **:** Social memory test 1(three chamber test)

SMT2 **:** Social memory test 2(Two trial direct interaction test)

SNpc **:** Substantia nigra pars compacta

SPT **:** Sucrose preference test

TH **:** Tyrosine Hydroxylase

TH+ **:** Tyrosine Hydroxylase Positive neuron

vCA1 **:** Ventral hippocampal Cornu Ammonis subfield 1

VH **:** Ventral hippocampus

VTA **:** Ventral tegmental area

YM **:** Y maze

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1. INTRODUCTION

Changes in the hippocampus have been described in many neurodegenerative and neuropsychiatric disorders where cognitive declines, including deficits in sociability and social memory, have been reported (1, 2). Even though the role of the hippocampus in processing and storing different types of memories have been studied for a long time, not much is known about the neural circuit and types of hippocampal neurons that control social memory (3).

Through our study, we will try to understand the role of catecholaminergic innervations in the dorsal and ventral parts of the hippocampus in social memory processing. We will achieve this through depleting the catecholamine from the terminals of the neurons using the neurotoxin 6-OHDA. Then we will combine both behavioral tests with tissue analysis to understand the mechanism of the associated behavioral deficit in our animals.

The next chapter will first discuss what social memory is and what the hippocampal subdivisions are. Then it will explore the current literature regarding the role of the hippocampus in social memory. Finally, it will explain the research aims and significance of our study, beside the possible limitations.

2. GENERAL INFORMATION

2.1. Social Memory

Social memory represents the cognitive and behavioral processes that include the ability of an individual to recognize a familiar or unknown conspecific, which is commonly known as social recognition. In addition, the ability to learn from others is so called social learning, which also could include the social memory (4). It is classified under the episodic type of memory, which we can recall and react to consciously(5).

Intact social memory is an important characteristic for the animals that live in social groups. It able to engage in a meaningful way in relationships with others. For example, social memory in rodents is critical to choosing proper social behaviors such as aggression, avoidance, cooperation, and keeping the social hierarchy. In addition, it could also be important for mating in some animals (5).

As research are ongoing to understand the neural circuit that controls this type of memory, different brain structures have been shown to be involved in social recognition and social learning, such as the olfactory bulb, the medial amygdala, the lateral septum, and recently the hippocampus and neocortex (4). Also, different neural pathways and subregions of these structures have been suggested for coding social memory that will be discussed later in this chapter.

Several neurochemicals have been suggested to play a role in social memory in different brain regions. One of these is oxytocin since studies show that injection of oxytocin or deletion of its receptor from hippocampus could affect social memory (6-9). Other substances such as vasopressin, norepinephrine, acetylcholine, dopamine, histamine, and glucocorticoid are also reported to be involved in the social memory processing (4, 7, 10, 11). However, their mechanisms of modulation and the affected structures by these substances are still hot research topics and further studies will be recommended.

2.2. Diseases Affecting Social Memory

Several neuropsychiatric and neurodevelopmental disorders manifest impairments in social behaviors and social memory, including schizophrenia and autism (12-14). Not only psychiatric disorders but also neurodegenerative diseases such as Parkinson's disease manifest social memory deficit as an early symptom of the premotor stage of the disease (15, 16). Patients with Alzheimer's disease also show signs of social withdrawal and a deficit in social memory (17). Furthermore, many animal models of these disorders manifest some social memory impairment phenotypes. As an example, the Shank2 knockout mouse model of ASD (18), the 22q11.2 deletion mouse model that has the genetic risk factor for schizophrenia (19), the A53T and 6-OHDA rodent models of Parkinson's disease (20, 21), and the APPswe/PS1 mouse model of Alzheimer's disease (17).

The absence of a complete understanding of human behaviour, such as sociability and social memory, and underlying pathology of neuropsychiatric and neurodevelopmental diseases, make it difficult to find a disease-modifying treatment for these illnesses.

2.3. Hippocampus

The hippocampus is an elongated anatomical structure, consists of two regions along the longitudinal axis: the dorsal and ventral regions, which are parallel to the human respective regions, the posterior and anterior hippocampus, as shown in (Figure 1.1). The hippocampus has been shown as a key region for the formation and retrieval of episodic memories and spatial navigation in the brain and also in emotions as part of the limbic systems (22).

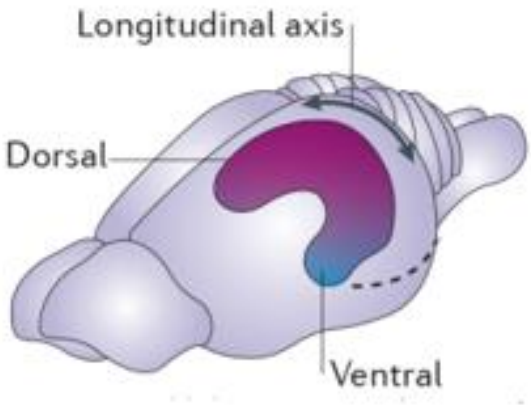


Figure 2.1. Dorsal and ventral hippocampus in the rodent brain from Strange *et al*.,2014.

2.3.1. Functions of Dorsal and Ventral Hippocampus

It has been argued whether the hippocampus's function is that same or distinct along its longitudinal axis. Some literature shows the distinct function between the dorsal and ventral hippocampus (23), while other show no absolute dissociation between them (24). Therefore, it is rationale to investigate whether the dorsal and ventral part of the hippocampus are overlapping or dissociate within each specific function.

Selective lesion on the dorsal hippocampus disrupted spatial memory, while lesion of the ventral pole spared spatial learning and had an anxiolytic effect (25). A specific role for the ventral, not dorsal, hippocampus in modulating anxiety-like behaviors in certain rat models that studying defense behaviors has been described by Pentkowski and his colleagues (26). In addition, it has also shown that, the ventral hippocampus could play a role in social interaction as the selective lesioning of it increased social interaction in some studies (27).

It has been suggested that the difference in function between the dorsal and ventral parts of the hippocampus could be due to the different sources of dopamine in the hippocampus. Dopamine (DA) released from the Locus coeruleus (LC) has been shown to play a role in spatial learning and memory in the dorsal hippocampus, while that released from the ventral tegmental area (VTA) into the ventral hippocampus

modulates contextual fear conditioning(28, 29). Not only LC and VTA that do innervate the hippocampus for DA, but also other dopamine sources have been discovered. For example, the midbrain raphe nuclei (RN), substantia nigra pars compacta (SNpc), nucleus accumbens (NAc) and their role in the hippocampus must be further investigated. These sources have been well illustrated by Elke Edelmann his colleagues as shown in (Figure 1.2)(28).

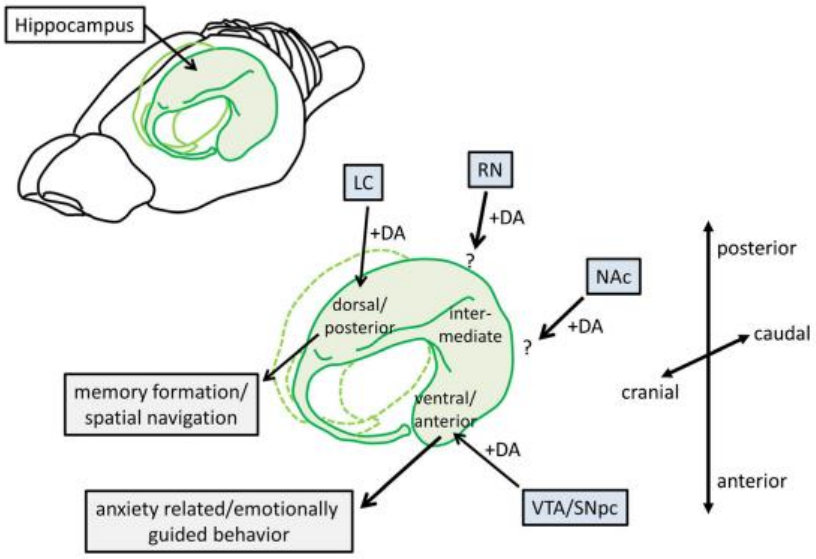


Figure 2.2. Different sources of dopamine and functional segregation of the hippocampus from Edelmann *et al*.,2018.

2.3.2. The Social Memory and Hippocampus

For a long time, the role of hippocampus regions in processing and encoding different types of memories has been studied. However, the role and type of hippocampal neurons involved in the neural circuit regulating social memory have not been well studied. The literature demonstrated that hippocampus lesion impaired social memory in rats, highlighting the importance of this structure in this type of memory (30). In addition, other brain regions have also been linked to playing a role in social memory in rodents, such as the olfactory bulb, prefrontal cortex, and insular cortex as well (31-33). It seems that these brain regions interplay and orchestrate the input and output of social memory as one network.

In the hippocampus the *Cornu Ammoni* sub-regions (CA) have been reported to be responsible for encoding and processing social memory. Dorsal hippocampal CA2 has been shown to encode social novelty and specific lesioning to this area produce social recognition impairment (34, 35). Not only CA2, but conditional deletion of dorsal hippocampal CA3 neurons led to the same impairment in social recognition and was suggested to have a role in storing and encoding social memory (8).

In a study by Okayama and his colleagues, who identified the ventral CA1 as a region for the storage of social memory, he recorded the activity of the ventral hippocampal CA1 region during the exposure of an animal to a familiar and novel animal. He shows that ventral hippocampal CA1 (vCA1) neurons of a mouse and their projections to NAc have more stable and strong firing when the mouse is exposed to a familiar mouse. This led them to conclude its role in the storage of social memory and to suggest a model for social information flow in the brain to encode social memory that is illustrated in (Figure 1.3) (36).

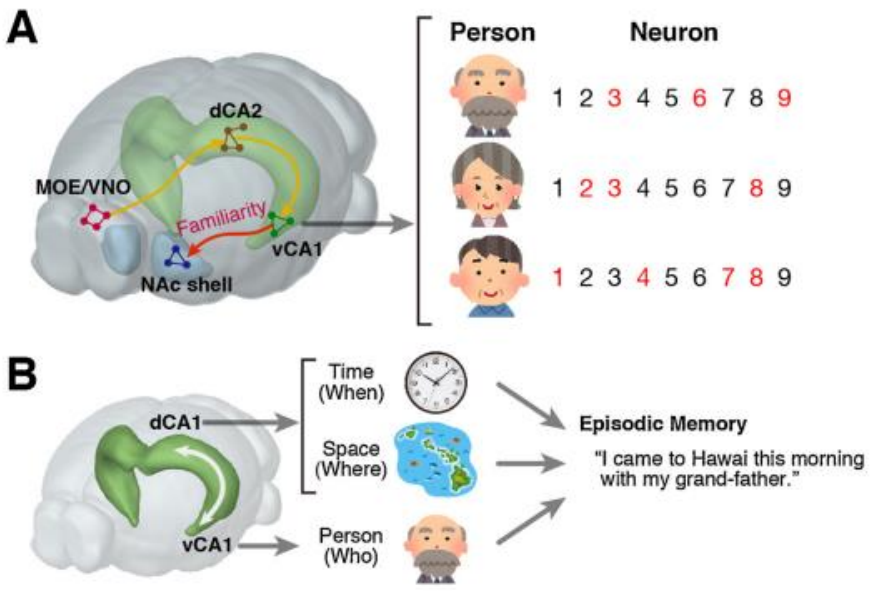


Figure 2.3. (A) Model of social information flows including "social engram pathway" in rodent (B) Functional distribution for representing a complete piece of episodic memory, Okayama *et al*., 2016.

In one study that conditionally deleted the oxytocin receptor from mice hippocampal CA2 and the distal part of CA3 (CA3a) which result in impairment of the long-term social recognition memory indicating the involvement of this receptor type. This was one of attempts to understand what molecular pathways could be included in the hippocampal regions during social memory formation(8). Also, activation of vasopressin terminals in the hippocampal CA2 region during memory acquisition could enhance social memory markedly. This effect could be diminished by vasopressin Receptor (V_{1B}) antagonist infusion (37). V_{1B} receptor that has been shown to be overexpressed predominately in the hippocampal CA2 region, rises interest in studying the exact function of the receptor pathways in the modulation of social memory (38).

In addition, neurotransmitter systems are also one of the major interests in this field. In a study done by Zinn and his colleagues showed that infusing β-adrenoreceptor agonist, isoproterenol, the D1/D5 dopaminergic receptor antagonist, SCH23390, and the H2 histaminergic receptor antagonist, ranitidine, into the CA1 modulates the long-term social recognition of the familiar juvenile mice (10). Although their localization could be questionable but still highlight the role that these neurotransmitters could play in social memory processing and the need for further specific study targeting their molecular pathway would be recommended.

2.4. Catecholamines

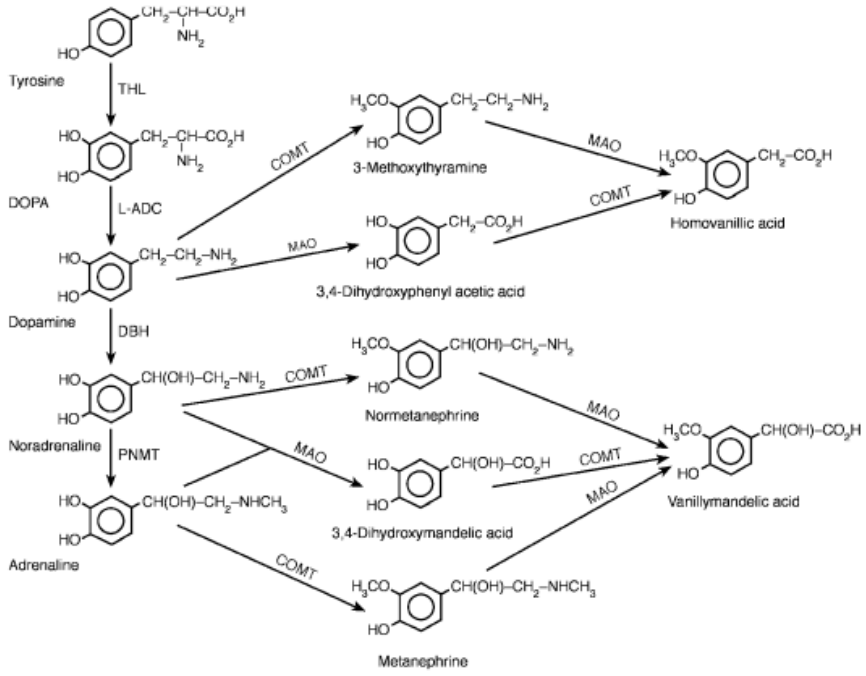
2.4.1. Synthesis, Storage and Metabolism

Noradrenaline, adrenaline, and dopamine, the clinically relevant catecholamines, are key neurotransmitters in the sympathetic nervous system that exert circulatory and metabolic effects by stimulating adrenergic receptors on a wide variety of cells. As chemical structure, they are monoamines attached to a benzene ring with two vicinal hydroxyl (catechol) groups.

Brain, adrenal medulla, and sympathetic neurons are the primary sites of catecholamine synthesis. Their synthesis requires hydroxylation of the amino acid L-tyrosine to DOPA, a rate-limiting step prior to the decarboxylation of DOPA to

dopamine, the primary central neurotransmitter in regions of the central nervous system involved in motor control. The addition of a β-hydroxyl group to dopamine by dopamine β-hydroxylase generates noradrenaline, while the addition of a methyl group to the amino group of noradrenaline generates adrenaline, significantly modifying the pharmacology of the catecholamine. In membrane-bound chromaffin granules of the adrenal medulla and in post-ganglionic neurons, catecholamines are retained chemically unaltered and stored until released (39-41).

The major mechanisms for the metabolism of catecholamines entering the circulation involve two enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), which result in a range of metabolites (41). The synthesis and metabolism of catecholamines is illustrated by Robert T Peaston and Cyril Weinkove in the following scheme (Figure 1.4).



Metabolism of catecholamines. COMT, catechol-O-methyltransferase; MAO, monoamine oxidase; THL, tyrosine hydroxylase; L-ADC, DOPA decarboxylase; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine N-methyltransferase.

Figure 2.4. Catecholamines metabolism from Peaston et al.,2004.

2.4.2. Catecholamines Function in Social Memory

Catecholamines have been extensively studied for different brain functions, including cognitive processes and memory. The literature on social memory reported

that activation of the norepinephrinergic system in central nervous system is involved in the memory for novel social stimuli, and NE depletion could lead to loss of discrimination of novel from familiar juvenile rats, supporting the evidence of norepinephrine modulation of social memory (42).

In addition, another study shows that specifically infusing norepinephrine reuptake inhibitor, nisoxetine, into the olfactory bulb improved the ability of rats to identify conspecifics. Similarly, the direct infusion into the NAc or the frontal cortex of D1 dopamine receptor agonist improves social recognition memory. However systemic injection of D1 dopamine receptor antagonist disrupts the social learning (10). These studies could prove the involvement of these neurotransmitter systems in social memory modulation.

The injection of 6-hydroxydopamine (6-OHDA) into striatum of rats, prevented the rat from recognizing a previously met juvenile rat in the second presentation, which demonstrates the impairment in the ability to recall the memory of social stimulus(21).

6-OHDA is a neurotoxic synthetic organic compound used to selectively destroy dopaminergic and noradrenergic neurons in the brain. One study has shown that this neurotoxin depletes catecholamine levels in the hippocampus (43). This depletion was reported to affect several cognitive processes, such as learning and taste memory in rats (44, 45).

Although the role of catecholamines has been studied in different brain regions, their role in the dorsal and ventral hippocampal modulation of social memory has not been studied. Therefore, in this study, our aim is to study the role of dorsal and ventral hippocampal catecholaminergic innervations in social memory by examining the effect of 6-OHDA on depletion of catecholamines in these regions. After that, we will evaluate cognitive and non-cognitive behaviors coupled with *in vitro* analysis to assess the biochemical change in targeted regions of the hippocampus.

Our study hypothesized that catecholamine depletion in the ventral and dorsal hippocampus could produce social memory impairment. In addition, we

hypothesized that, the depletion could affect the other types of memory, such as recognition and spatial memory in the dorsal group, while it could produce anxiolytic effect in the ventral group.

The importance of the study is that they will highlight the role of catecholaminergic terminals in the hippocampus in sociability and social memory. This could lead to a better understanding of the neuropathology of many psychiatric and neurodegenerative diseases that manifest the sociability and social memory impairment. Furthermore, it could provide a valid animal model to screen drugs for symptomatic treatment of these disorders.

3. MATERIALS AND METHODS

3.1. Animals

Female Wistar rats (220–320g) were used in this study. Animals were kept at a controlled room temperature (20±2°C) in groups of 3-4 animals per standard Plexiglas cages. The animals were kept in a 12-12-h light/dark cycle. All animals were given *ad libitum* access to water and food throughout experiments. All operations were conducted in compliance with the Hacettepe University, Guidelines for the Care and Use of Laboratory Animals (Ethic approval of Hacettepe University under the code:2022/01-09). Special efforts were made to minimize animal suffering and reduce the number of animals utilized to the minimum number that necessary for statistical accuracy.

3.2. Stereotaxic Surgery

The stereotaxic coordinates for the study have been optimized using methylene blue dye followed by direct brain dissection using microtome cryostat-(MC5050, Italy) until the injection coordinates were successfully localized in either the dorsal hippocampus (DH) or ventral hippocampus (VH) for respected groups. The coordinates for DH stereotaxic injections -2.8 mm posterior to the bregma (AP), ±1.8 mm from the midline (ML), and 3.8 mm ventral from the surface of the skull (DV), whereas the coordinates for VH injections -5.2 mm posterior to bregma, ±5.3 mm from the midline, and 6.0 mm ventral from the surface of the brain (Figure 3.1).

Animals were anesthetized with intraperitoneal injection of anaesthesia combined ketamine 100mg/kg (Keta-Control®, Dogal ilac) and Xylazine 20 mg/kg (Xylazinbo%2®, Intermed) (as a ratio 9:1). The forelimb and hindlimb pedal withdrawal reflex were used to assess the depth of anaesthesia before starting the procedure. After the animal deeply anesthetized, it placed over a plastic bag filled with hot water in the Stereotaxic Instrument (Kopf, USA) (Figure 3.2). The upper incisor bar was positioned 3.3 mm below the interaural line, and the skull was levelled, ensuring the same DV reading of bregma and lambda.

A drill was used to make small hole in the skull. The skull was cleaned with water and cotton while drilling to remove any remaining particles. Then, animals received bilateral injections of 6-OHDA (in ascorbic acid that is dissolved in 0.9% saline to make solution of 0.02% ascorbic acid) or sham per hemisphere in either DH or VH using Hamilton syringe of 5 µl size. The injected volume was 0.5 µl contain 6 µg of 6-OHDA in each hemisphere at a rate of 0.5 µg/min. After injecting the 0.5 µl, the syringe was left in its place for 2 minutes, then elevated 1 mm up and left for another 2 minutes before being carefully withdrawn from the skull. There are two separated syringes were used for either the sham or the toxin for each group and the syringes was cleaned with hydrogen peroxide followed by water to remove any remaining blood they could attached during the injections. During the surgery, animal has been monitored and their eyes were kept moisturized using saline.

After the injection, the animals were sutured with silk suture (USP 4/0, Dogsan®) and covered with povidone to prevent infection. The animals received a normal saline injection of 1 ml subcutaneously to prevent dehydration through the recovery period, then 0.7 ml of paracetamol (10 mg/ml) was injected intraperitoneally to reduce the pain and suffering.

To maintain an animal's body temperature in a normal range and to prevent hypothermia, animals were placed on the homeothermic table until they recovered from anaesthesia. The animals were only placed back in their home cage after a full recovery to prevent any aggressive attacks from other cage mates.

(A) (B)

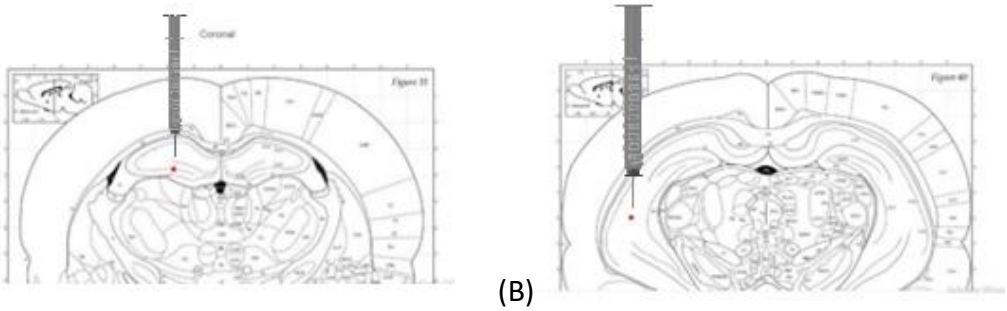


Figure 3.1. Dorsal (A) and ventral hippocampus (B) injection coordinates.

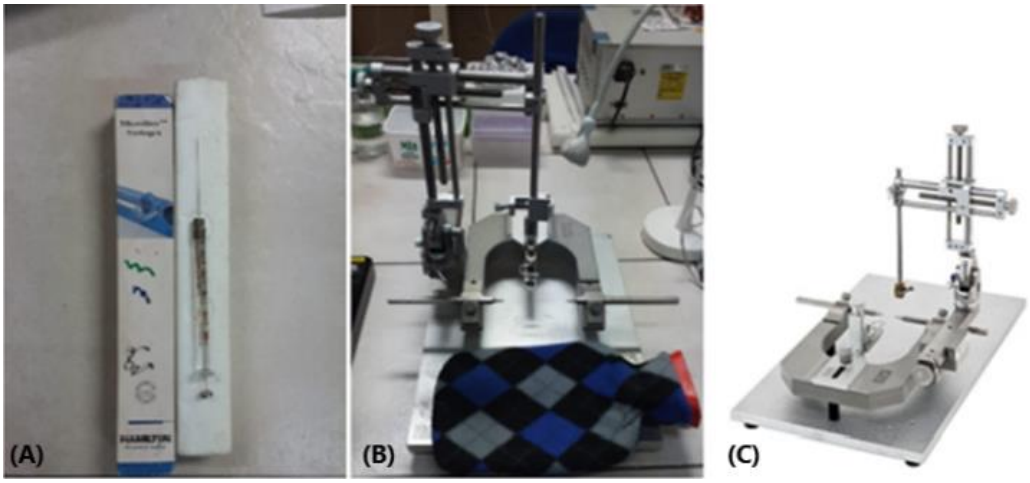


Figure 3.2. (A) Hamilton syringe, (B) Stereotaxic surgery setup, and (C) Stereotaxic instrument.

3.3. Experimental Groups

The experimental groups used in the study and the number of animals in each group are described in Table 3.1.

Table 3.1. Experimental groups and the number of animals for each group

Groups	Number of animals	Abbreviation
Animals injected with 6-OHDA in the dorsal hippocampus	10	Dorsal
Animals injected with 6-OHDA in the ventral hippocampus	10	Ventral
Animals injected with the sham in the dorsal hippocampus	8	Sham-D
Animals injected with the sham in the ventral hippocampus	8	Sham-V
Naïve animals	8	Naive

3.4. Behavioural Tests

Animals were monitored for any sign of infections or serious body wight loss for about 10 days following the stereotaxic injections. The animal weight was monitored throughout 4 weeks from the surgery. No serious body loss has been observed as shown in Figure 3.3.

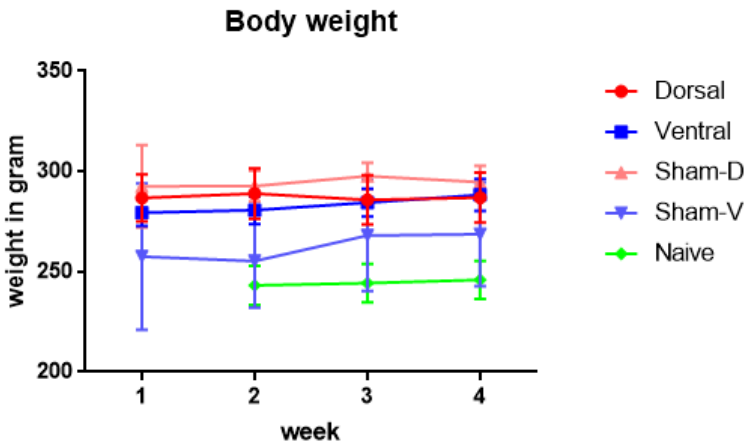
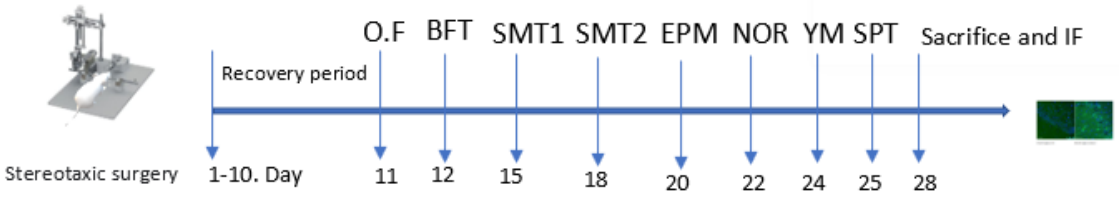


Figure 3.3. Body weight monitoring curve.

The behavioural tests were started 10 days after the surgery to ensure animals recovery. Then the animals were habituated to experimenter by handling and to the test room for 3 days before starting the behavioural tests. One-day off was set between some experiments to allow the animals to rest and to minimize the stress. To reduce the variation, all experiments were performed by the same experimenter, and all tests were planned to be performed at the same time in the day for all groups as possible. The used schedule was described in Figure 3.4. The behavioural tests were described in detail as below:

(A)



(B)

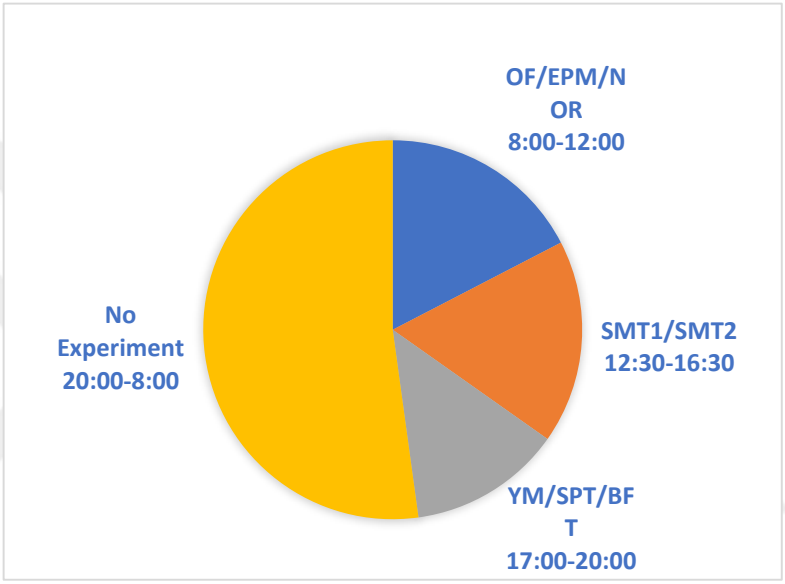


Figure 3.4. The study scheme (A) The experimental scheme (B) Time chart for the behavioral test, OF: Open filed, SMT1: Social memory test 1(three chamber test), SMT2: Social memory test 2 (Two trial direct interaction test), BFT: Buried food seeking test, NOR: Novel object recognition test, YM: Y maze, SPT: Sucrose preference test, EPM: Elevated plus maze test.

3.4.1. Open Field Locomotor Activity

The test was performed on the 11th day after the surgery. The locomotor cages, which is used in these experiments, are plexiglass boxes (40 × 40 × 40 cm) designed with infrared photo beam emitters and sensors (Figure 3.5) to detecting animals vertical and horizontal activity. This was applied to assess whether the dorsal and ventral hippocampal catecholamine depletions have any effects on locomotor activity.

Figure 3.5. Open field locomotor activity cages.

On the testing day the animals were transferred to the testing room for 40 minutes before the tests for habituation. Then, the rats were placed in locomotor activity cages and their activity was recorded for 30 minutes. The total distance moved (cm), ambulatory, vertical, stereotypical, and horizontal activity were measured automatically using locomotor activity analysis system (Activity meter, ACT508, Commat Ltd., Turkey). The measured activities were used to compare the experimental groups.

3.4.2. Buried Food Seeking Test

The test was performed on the 12nd day after the surgery to assess the effect of dorsal and ventral hippocampal catecholamine depletions on olfaction, as it is the main sensory modality for social memory in rodents. Animals is food restricted for 8 hours in their home cages, with free access only to water. The test was conducted in a standard cage similar to their home cage and their daily pellets were used during the test to prevent novel food-induced neophobia that could affect normal olfaction behaviour. The animals were allowed to habituate to the testing room for 60 minutes prior to the test. Before placing the animals inside the standard cage for testing, a standard pellet equal to 2.00 g was buried 0.5–1 cm under the 3cm height bedding as it is showed in (Figure 3.6). The bedding and the position of the pellets was changed between each test session, which was preceded by cleaning with 80% ethanol, followed by distilled water, and dry clean tissue. The experimenter was

changing gloves between tested animals to prevent any unintended odour transfer. All experimental group has been tested in the same cages.

In the test session, the tested animal was placed inside the standard cage facing the wall that is far from the buried pellet. The time to find (pickup-consume-uncover) the pellet was calculated using a stopwatch by an experimenter standing outside the testing room. The animal who failed to find the pellet throughout 10 minutes, their latency determined as 10 minutes only if the animal was able to go towards the exposed pellet. Animals that failed to find the pellet in 10 minutes and then showed no interest in the exposed pellet were excluded from the experiment, as they were not motivated by the food initially rather than olfactory dysfunction.

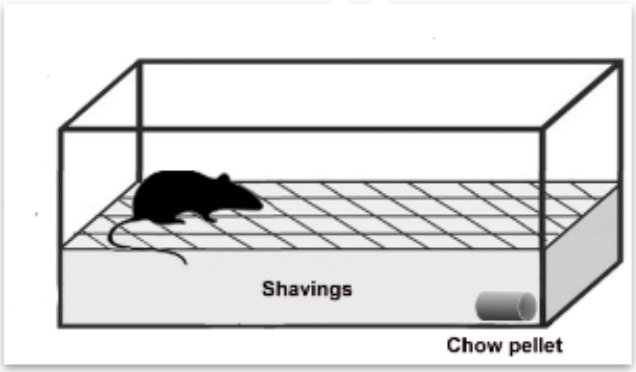


Figure 3.6. Test setting for Buried food seeking test, from Cleiton *et al.*,2018.

3.4.3. Social Memory Tests

To assess social memory, two tests have been conducted: the three-chamber social memory test for assessment of short-term social memory and the two-trial direct interaction test for long-term social memory. The details of each test have been described below.

3.4.4. A. Three- Chamber Social Memory Test

The three-chamber social memory test is a sensitive and reliable behavioural test for the assessment of sociability and social novelty preference as a parameter for measuring social deficits and social memory in several rodent models of disease (46).

Most social memory studies used mice. However, in our study, we preferred to use rats instead of mice due to some advantages in terms of social behaviour. For example, rats are social animals that get less stressed by human contact and have less latency to engage in social interaction compared to mice (47). In addition, they have a larger brain compared to the mice, which will facilitate targeting small brain regions. Therefore, we decided to optimize the mice protocols for the three-chamber social memory test to be used for the Wistar rats.

There are several different adapted protocols for this test. So, before applying the experimental groups we made an optimization trial. For optimization, a group of naïve Wistar rats (n = 10, 220-230g) has been used and different experimental protocols (including different session lengths, different stimuli (juvenile, adult), and different cylinder diameters) have been used until an optimized protocol which produced a replicable and reliable results was finalized for the experiment.

The Optimized Protocol:

The apparatus is a black box of 90 L x 50 W x 40 H cm made of wood and divided into three chambers (left, middle, and right) of 30 x 50 cm each. These chambers are interconnected with a small door of 12 x 10 cm that allows the animals to explore the apparatus. The left and right chambers contain cylinders to hold the stimulus animal. The cylinder is 20 cm high and 13 cm in radius and has a wall that was made of iron bars with 1.4 cm inter-bar space. The apparatus was shown in (Figure 3.7).

Figure 3.7. Three-chamber social memory protocol and apparatus (the left cylinder was laid for the demonstrative purpose only).

The test was performed on the 15th day after the surgery in a low-light room, and auditory and visual cues have been reduced to the minimum. Before the test day, both the animals and the juvenile stimulus used in the test have been habituated to the testing room for 1 hour. Then, inside the apparatus for 20 minutes to reduce the stress associated with a novel environment before the test day. On the test day, the animals were placed inside the testing room for 40 minutes to allow the animals to recover from the stress that could be induced by the transfer. Then, the test protocol was performed, which consists of three sessions.

In the first session, chamber preference, the animals were allowed to freely explore the apparatus and the cylinders for 5 minutes. In the second session, sociability session, a juvenile rat 1 (gender and strain matched) was placed inside the cylinder in either the left or right chamber (systematically alternated) and the other cylinder was left empty. Then the animal was placed in the middle chamber and allowed to investigate for 10 minutes, which called sociability session. Then, directly the third session is social novelty, which started by placing a novel juvenile female rat 2 (gender and strain matched) in the empty cylinder in the other chamber, and the first juvenile rat 1(familiar one) from the previous session remained in its place, and the animal was allowed again to investigate for 10 minutes. A stock of familiar and

novel juvenile rats has been maintained during the test. This is to allow testing of a larger number of animals without causing stress for them which could occur if only one novel or familiar rat was used to test all the animals. Also, care has been given to the housing of animals throughout the study; therefore, the novel and familiar rats have been housed in different cages as far from the tested animals as possible to prevent any animal odour familiarization before the test.

The apparatus was cleaned using tissue wetted with ethanol 70%, followed by distilled water and dry tissue to remove any olfactory cues that could interfere with the test outcome. Furthermore, the experimenter changed his gloves continuously between the trials to prevent any unintended odour transfer.

All the sessions have been recorded using the automated video tracking system (TSE VideoMot2, Germany), and the time spent in each chamber, and the number of visits, were calculated for each session. The protocol and apparatus were illustrated in (Figure 3.8).

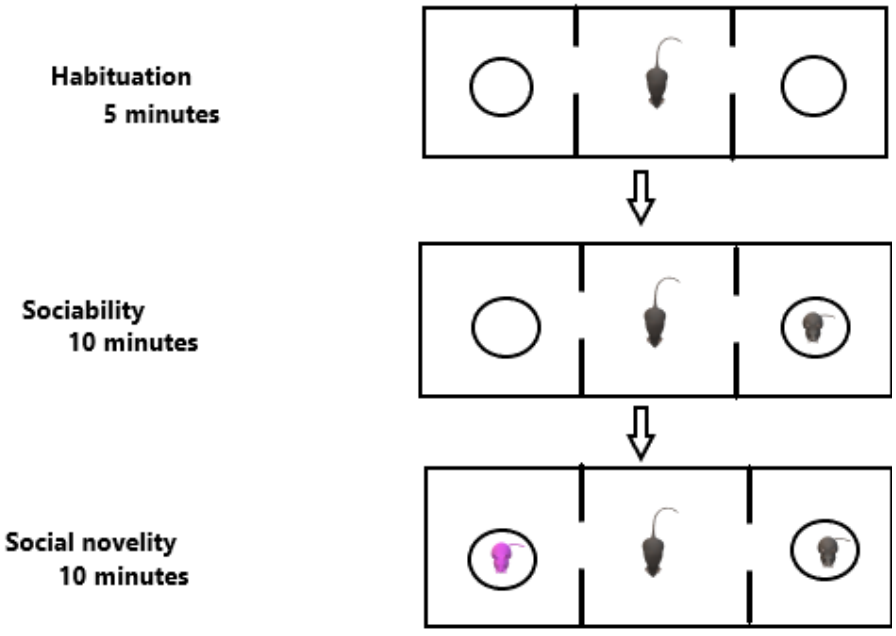


Figure 3.8. The schematic representation of the three-chamber social memory test protocol.

3.4.5. B. Two-Trial Direct Interaction Test

The test was performed on the 18th day after the surgery to assess the long-term social memory. The protocol was also optimized based on the pilot study conducted in the same rats used for three chamber protocol optimizations until replicable and reliable results obtained.

The test was performed in the behavioural room with a neutral environment in a non-transparent, black open-field arena. The habitation phase has been conducted for all animals one day before the test day for 40 minutes for the room and five minutes for the test apparatus. On the test day, the animal was allowed for 30 minutes to become habituated to the test room and recover from the minor anxiety that might be caused by the transfer of the animal from the animal facility to the behavioural room.

The test started with trial 1, in which the animal was placed inside the arena for 5 minutes for habituation. Then, session 1 of trial 1 was started for 5 minutes in which the animal was allowed to interact with a stimulus novel juvenile rat 1 (gender and strain matched) and the experimenter recorded the social interaction time initiated only by the test animal using a stopwatch. At the end of session 1, the stimulus animal was removed from the arena and placed back in its holding cage. After a 1-hour interval, the animal was allowed to interact with the previous met stimulus in session 2 for another 5 minutes (rat 1), and the time of interaction was recorded again.

In trial 2, the two sessions were performed similarly to those of trial 1. However, in both sessions, a newly not met juvenile rats (gender and strain matched) were introduced (rat 2, rat 3).

The whole experiments were recorded with a video system above the arena, and the interaction times were calculated using a stopwatch by a trained experimenter. Social interaction is defined as: sniffing (anogenital, nose-nose), following a stimulus animal within 2 cm, and allogrooming. The social interaction was recorded only when initiated by the test animal, and the recording continued as long as no aggressive behaviour was encountered during the 5-minute trial. In case of any

aggressive behaviour during the test session, this would lead to the exclusion of the animal from data analysis and the discontinuation of the test for this animal. In this experiment, there was no such behaviour observed due to the use of juvenile rats as a stimulus. The apparatus was cleaned using tissue wetted with ethanol 70%, followed by distilled water and dry tissue to remove any olfactory cues that could interfere with the test outcomes. Furthermore, the experimenter kept changing his gloves between the trials to prevent any unintended odour transfer. The protocol and apparatus were illustrated simply in Figure 3.9.

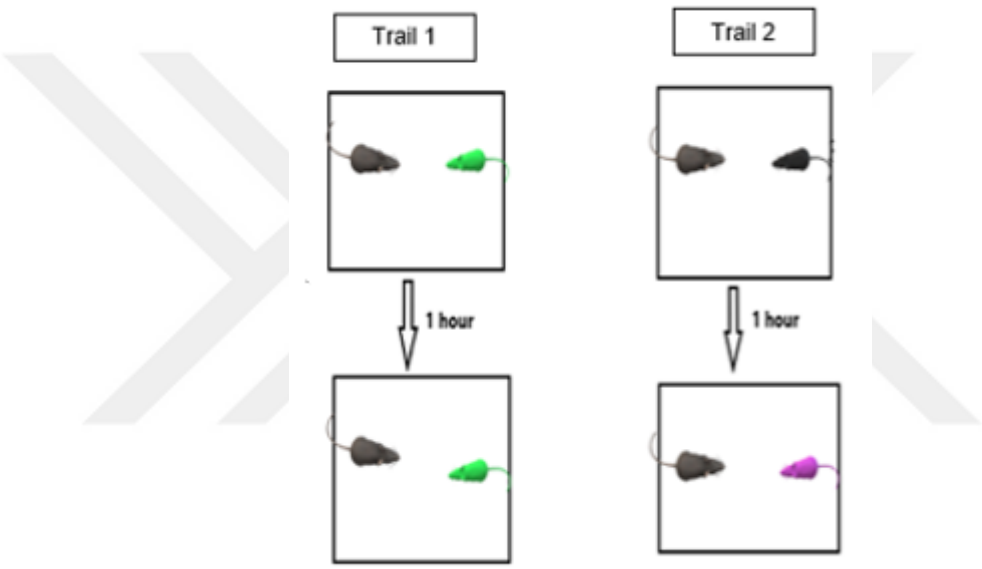


Figure 3.9. The schematic representation of the two-trial direct interaction test protocol.

3.4.6. Elevated Plus Maze (EPM)

For evaluation of anxiety-like behaviours, the EPM test was conducted on the 20th day after the surgery, using a plus-shaped plexiglass apparatus, black in colour, and consists of four elevated arms (L x W x H = 45 x 12 x 70 cm). Two of the four arms have high walls of 45 cm and are opposite to each other (closed arms), whereas the other two arms are open (open arms). Between the four arms, there is a center squire of 12 x 12 cm that works as a bridge as it is seen in (Figure 3.10). The only source of light is the desk lamp, to keep the room dim and prevent any anxiety that could be triggered by the light.

The animals were allowed to habituate to the test room for 30 minutes before the test. The test started by placing the animal in the center square where it faced the closed arm. Then, the animal was released, and its behaviour were tracked using a video tracking system (TSE VideoMot2, Germany). The amount of time spent and the number of visits to each arm were reported. The final score was calculated and compared between the experimental groups as follows:

$$\% \text{ Time spent in the open arm} = \frac{(\text{Time spent in the open arms})}{(\text{Time spent in the open arms} + \text{closed arms})} \times 100$$

Figure 3.10. Elevated Plus Maze (from the front top view).

3.4.7. Novel Object Recognition Test (NOR)

The animals were evaluated on the 22nd day after the surgery for recognition memory by using NOR test. The test conducted in black box of 40 x 40 x 40 cm and

the animals were observed through camera system for video tracking (TSE VideoMot2, Germany).

Before the testing day, the animals were habituated to the room for 40 minutes and for the apparatus for 10 minutes. On the test day, the animals were undergone two testing phases, the first was familiarization phase in which two identical objects (two same colour and size) were placed inside the box, both with distance of 10 cm from the front box wall compared to the experimenter. After that, the animals were placed in the box facing the wall which was close to the experimenter that far from the object and then were allowed to investigate the object for 10 minutes. After 1 hour, one of the objects was replaced with novel object (different colour, size, and shape) and placed at the same location with the one of the previous objects (systematically altered). Then, the animals were placed again inside the box facing to the wall and allowed to investigate the objects for 5 minutes as a testing phase. The time spent with familiar and novel object were detected and analyzed. The score was calculated as follow:

$$\text{Discrimination index} = \frac{\text{(Time spent with novel object)}}{\text{(Time spent with novel object + familiar object)}} \times 100$$

Between the trials, both the box and the objects were cleaned with 70% alcohol followed by distil water, then dried with tissue to eliminate any olfactory stimuli. In addition, the place of the novel and familiar object was alternatively changed for each set of animals to prevent any side preferences.

3.4.8. Y-Maze

The Y-maze was performed on the 24th day after the surgery for evaluating the short-term spatial memory in rodents. The apparatus is made of wood and black in colour. It consists of three arms in the shape of the letter "Y". Each arm is 50 x 10 x 20 cm and has an angle of 120 between the arms (Figure 3.11).

Figure 3.11. Y-maze apparatus.

Spontaneous alternation is a measurement for spatial working memory, and it can be evaluated by enabling rodents to explore all three arms of the Y maze. This is facilitated by the intrinsic desire of rodent to investigate previously unexplored regions. A rodent with intact working memory functioning, will recall the previously visited arms and exhibit a preference for entering a new arm. Spontaneous alteration is calculated when the animal visits the 3 arms in a consecutive manner.

On the test day, animals were transferred to the behavioural rooms and allowed to habituate for 40 minutes. After that, the animal was placed at the end of one arm of the Y maze and allowed to investigate for 5 minutes. Spontaneous alternations, the percentage of the alternation, and the number of visits to previous, same, and new arms were recorded using automated video tracking system (TSE VideoMot2, Germany).

3.4.9. Sucrose Preference Test

The test was performed on the 27th-28th day after the surgery to assess anhedonia, which is one of the core symptoms of depression. Rodents have a natural tendency to consume sweet foods over non-sweet ones. The ability to experience pleasure is called hedonia, and it could be affected if the animal experiences a state of depression (48).

The test protocol has been adapted from our previous study (49). Animals were kept in individual cages during the test, then each cage was provided with a standard bottle of 2% sucrose solution for 24 hours to habituate the animal and prevent neophobia. Then, animals were restricted from water for 8 hours. After that, each animal as provided with 2 bottles: one contained 2% sucrose solution and the other contained tap water. The location of the bottle changed every 8 hours to prevent side bias preference. The test continued for 24 hours and then the volume consumed from each bottle was calculated and the result expressed as a percentage of sucrose preference as follows:

$$\text{Sucrose Preference Percent} = \frac{\text{(sucrose solution consumption)}}{\text{(sucrose solution consumption + tape water consumption)}} \times 100$$

3.4.10. Histological Analysis

The histological analysis was conducted to evaluate changes in catecholamine level in the dorsal and ventral hippocampus and to determine if the observed behavioural abnormalities were attributed by the depletion of catecholaminergic terminals in hippocampus.

3.4.11. A. Brain Isolation

At the end of the behavioural experiments, the animals were perfused transcardially for immunofluorescence staining on the 28th day. Animals were anaesthetized with a high-dose ketamine/xylazine mixture (9:1, IP). After the animal was deeply anesthetized, the chest was exposed with an incision to skin using dissecting scissors, followed by the diaphragm and ribcage. After exposing the heart, the needle connected to the perfusion pump (MasterFlex Console Drive®, USA, Figure3.12.A) was inserted into the apex of the left ventricle, then a small cut to the right atrium was made with scissors to allow the drain of blood from the circulation. For 2 minutes, animals were perfused with 30% heparin to prevent clotting, then with

4% paraformaldehyde for 4 minutes for reperfusion and tissue fixation (flow rate of 40 ml/m). After reperfusion, the brains were surgically removed and incubated in 4% paraformaldehyde solution for 24 hours in the refrigerator at +4°C, then transferred to a 30% sucrose solution for long-term cryoprotection and kept in the refrigerator at +4°C.

3.4.12. B. Brain Dissection

Coronal sections were taken using cc at a 35 µm thickness (Figure 3.12.B, C), after the brain was covered with the freezing gel (Tissue-Tek®O.C.T., USA) and stabilized to the holder. The sections were placed sequentially in 24-well plates contains antifreeze solution of two salts, sodium phosphate monobasic (13.75 g/L) and sodium phosphate dibasic heptahydrate (25.75 g/L) dissolved in mixture of distilled water, ethylene glycol and glycerol (4:3:3). After that, the section was kept in - 80°C until immunofluorescence staining.

(A) (B) (C)

Figure 3.12. (A) Perfusion pump, (B) MC5050 microtome Cryostat and(C) Brain position.

3.4.13. C. Immunofluorescence Staining

Sections were selected for both the dorsal and ventral hippocampus regions and transferred to the one hole of the 24-well plate for the single antibody staining. All shams, DH, and VH groups tissue of animals were stained simultaneously. Firstly, the selected sections were washed with PBS solutions to remove remaining antifreeze solution. Then for permeabilization, the sections were rinsed twice with 0.1% triton X-100 in PBS (PBS-t) for 5 min each. After that, the sections were

incubated with 10% normal goat serum (NGS) in PBS-t for 1 hour over the shaker at room temperature. Then, the samples were incubated with tyrosine hydroxylase primary antibody (ab75875, 1:600, Abcam, UK) in PBS-t and 10% NGS overnight at room temperature. On the second day, the sections were rinsed 3x10 minutes with PBS-t, then incubated with fluorochrome-conjugated secondary antibody (Goat anti rabbit, 1:200) in PBS-t and 10% NGS for 90 minutes. After that, the samples were rinsed 2x5 minutes with PBS-t, then 1x5 minutes with PBS. After fixing the sections to the poly-L-lysine coated slide, the sections were allowed to semi-dry, then covered with 20 µl of Hoechst dye (to staining the nuclei) and closed with lamella. The stained sections were kept after covering with aluminum foil at -20°C until they were analyzed with confocal microscopy.

3.5. Statistical Analysis

All statistical analysis has been done using the GraphPad Prism programme (GraphPad Software V7, Inc., San Diego, CA, USA). For comparisons of two groups or two data sets inside one group, unpaired Student's t tests were selected, while for comparisons of more than two groups, one-way analysis of variance (ANOVA), multiple comparisons were performed. Results were expressed as mean ± standard error of mean (SEM), and a p-value less than 0.05 was considered to be statistically significant

4. RESULTS

4.1. Effect Of Dorsal and Ventral Hippocampal Catecholamine Depletion on Locomotor Activity

In open field test, the horizontal, vertical, ambulatory, stereotypic activities, and the distance travelled were evaluated in all experimental groups. The depletion of catecholamine in the ventral group (VH) increased the ambulatory ($p=0.0046$ VH vs Naive, Figure 3.1.A), horizontal ($p=0.022$ VH vs Naïve, Figure 4.1.B) movements, and the distance travelled ($p=0.0127$, VH vs Naïve Figure 3.1.C). Whereas it did not affect the vertical and stereotypic activities of the VH ($p >0.05$, VH vs Naive Figure 4.1.E, D). Regarding the dorsal group (DH), sham ventral (Sham-V), and sham dorsal (Sham-D) groups, no changes in locomotor activity have been observed ($p >0.05$, Naïve vs DH, Naïve vs Sham-D, Naïve vs Sham-V, Figure 4.1).

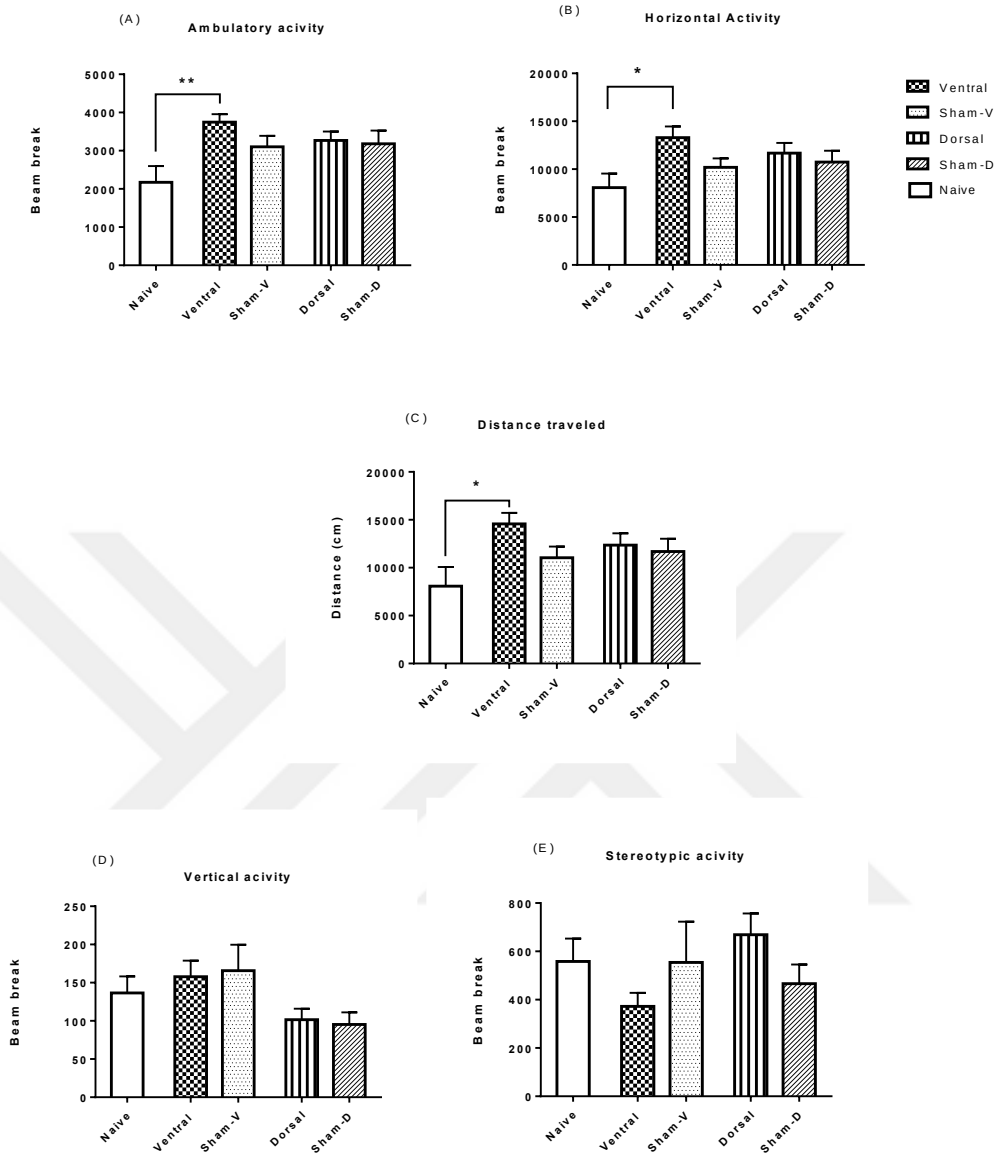


Figure 4.1. Effect of dorsal and ventral hippocampal catecholamine depletion on locomotor activity. (A) Ambulatory (**p=0.0046 VH vs Naïve), (B) Horizontal (*p=0.022 VH vs Naïve) (C) Distance traveled (*p=0.0127 VH vs Naïve) (D) Vertical activity (E) stereotypic. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; one-way ANOVA, Multiple comparisons, Data expressed as Mean ± SEM).

4.2. Effect Of Dorsal and Ventral Hippocampal Catecholamine Depletion on Olfaction

In buried food test (BFST) all groups were evaluated for olfaction as it is an important sensory modality for social memory in rat. The ability to smell and find

hidden pellets was used for the assessment of olfaction. In this test there is no difference in the latency to find pellets between VH, DH, Sham-V, Sham-D, and naive groups which could indicated the lack of effect on olfaction after the depletion of catecholamine in dorsal and ventral hippocampus (Figure 4.2).

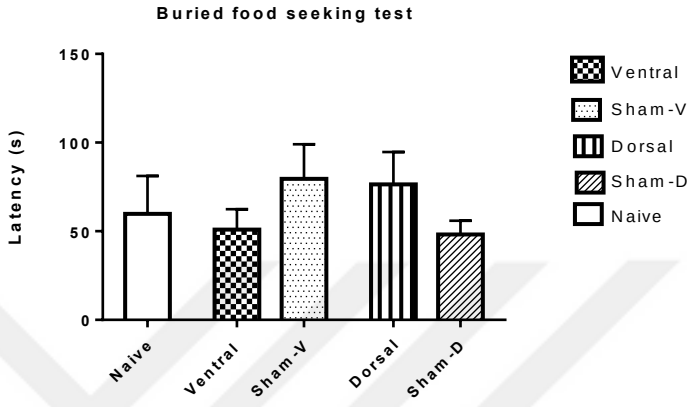


Figure 4.2. Effect of dorsal and ventral hippocampal catecholamine depletion on latency to find pellets in BFST. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; one-way ANOVA, Multiple comparisons, Data expressed as Mean ± SEM).

4.3. Effect Of Dorsal and Ventral Hippocampal Catecholamine Depletion on Social Memory

The social memory tests were conducted for all the experimental groups. To assess short- term social memory we used three-chamber social memory test and for the long-term social memory we used two-trial direct interaction test.

4.3.1. Three Chamber Social Memory Test (3-CSMT)

In the first session of the test, chamber preference session, no difference was found between number of visits, or the time spent in left and right chamber of the apparatus in VH, DH, Sham-D, Sham-V, Naïve groups. This could indicate the absence of side bias ($p > 0.05$ time spent in right vs time spent in left chambers, number of visits to right vs visits to left chamber, Figure 4.3).

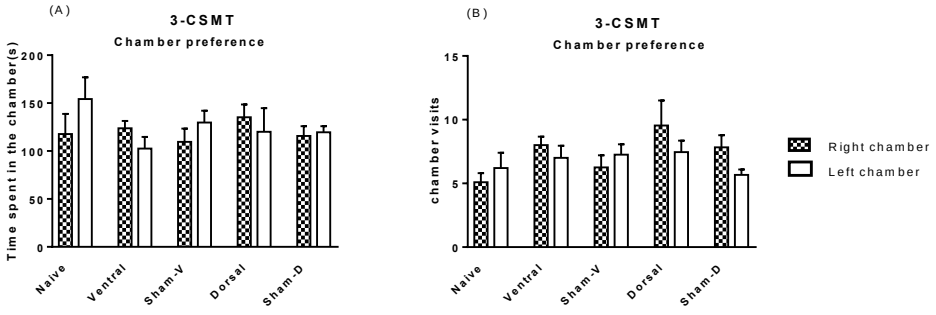


Figure 4.3. Effect of dorsal and ventral hippocampal catecholamine depletion on chamber preference in 3-CSMT. (A) Time spent in the chambers (B) Number of visits to each champers in 3-CSMT. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; unpaired t test, Data expressed as Mean ± SEM).

During the sociability session VH group did not show preference for the rat containing chamber compared to the empty one, whereas DH, Sham-D, and naive groups preferred the chamber containing rat compared to the empty chamber (Sham-D **p=0.0074, Naive***p=0.0006, Dorsal****p<0.0001, time spent in rat's chamber vs time spend in empty chamber, Figure 4.4). Notably, Sham-V shows a tendency and preference to spent time with other rat, but it did not reach statistical significance.

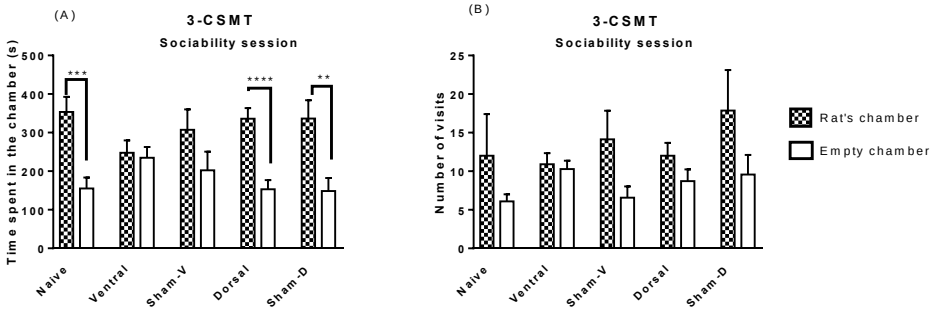


Figure 4.4. Effect of dorsal and ventral hippocampal catecholamine depletion on Sociability in 3-CSMT. (A) Time spent in each chamber (Sham-D **p=0.0074, Naive***p=0.0006, Dorsal****p<0.0001, time spent in rat's chamber vs time spent in empty chamber) (B) Number of visits to each chamber. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; unpaired t test, Data expressed as Mean ± SEM).

In the social novelty session, both DH and VH groups were unable to discriminate between a familiar and novel juvenile rat so they spent similar time in both chambers (Figure 4.5), while Sham-V, Sham-D, and naive groups were able to recognize them and they spent more time in the novel rat's chamber (Sham-V *p=0.0190, Sham-D **p=0.0089, Naïve ***p=0.0007, time spent in Familiar rat's chamber vs time spent in novel rat's chamber, Figure 4.5).

Taking the number of visits to each chamber in every session, it shows a similar pattern as time spent in each chamber, however, it did not reach statistical significance (Figure 4.3.B, 4.4.B, 4.5.B).

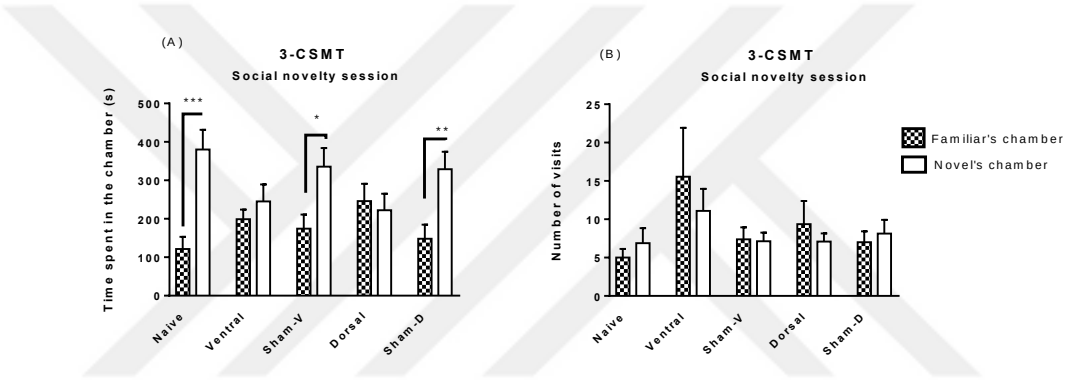


Figure 4.5. Effect of dorsal and ventral hippocampal catecholamine depletion on social novelty in 3-CSMT. (A) Time spent in each chamber (Sham-V *p=0.0190, Sham-D **p=0.0089, Naïve ***p=0.0007, time spent in Familiar rat's chamber vs time spent in novel rat's chamber) (B) Number of visits to each chamber. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; unpaired t test, Data expressed as Mean ± SEM).

4.3.2. Two Trial Direct Interaction Test (2-TDIT)

In 2-TDIT, Sham-V, Sham-D, and naive groups were able to identify previously met juvenile rat 1 (Sham-V ***p= 0.0001, Sham-D ****p <0.0001, Naïve ****p <0.0001, time of interaction in the first session vs time of interaction in the second session, Figure 4.6.A). However, the DH group failed to recognize a previously met juvenile rat 1 as no difference was observed between times of interaction with same juvenile exposed twice in trial 1 (Figure 4.6.A). Additionally, no reduction was observed in the interaction with two novel juvenile rats (rat 2 and 3) exposed in trial 2 (Figure 4.6.B) which is not due to deficit in sociability. The VH group also showed a

reduction in time of interaction with same juvenile rat 1 exposed twice in trial 1 (p=0.0028, time of interaction in the first session vs time of interaction in the second session, Figure 4.6.A). However, a similar reduction was observed again when exposed to two novel juvenile rat 2 and rat 3 in trial 2 (p= 0.0499, time of interaction with novel rat 2 vs time of interaction with novel rat 3, Figure 4.6.B).

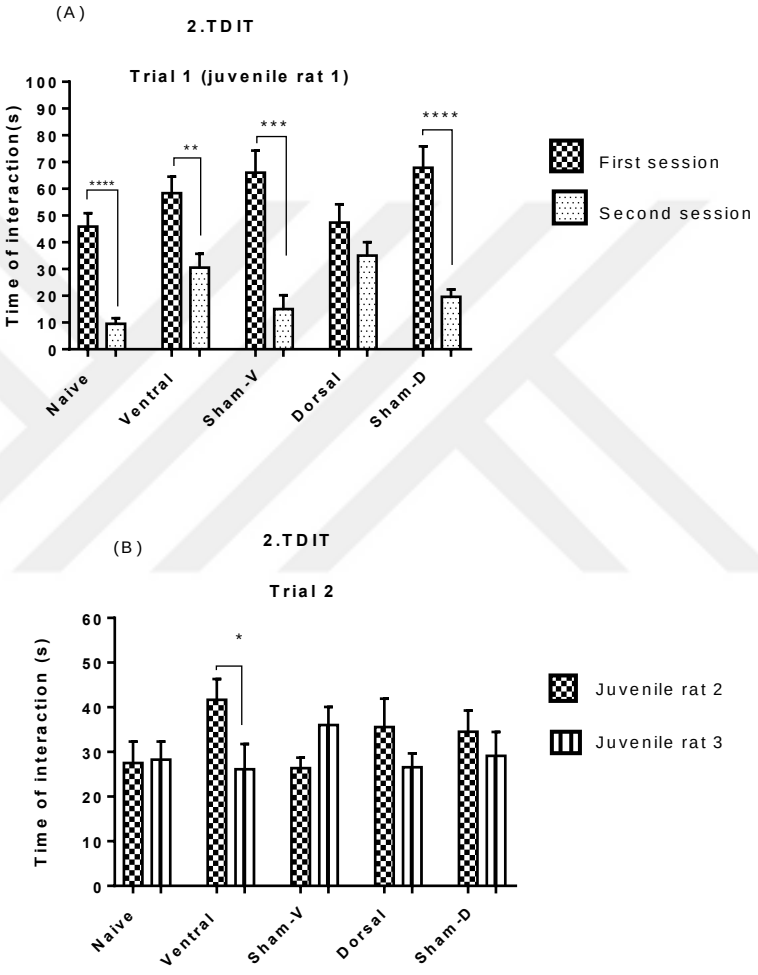


Figure 4.6. Effect of dorsal and ventral hippocampal catecholamine depletion on interaction time in 2-TDIT. (A) Trial 1 (VH **p=0.0028, Sham-V ***p= 0.0001, Sham-D ****p <0.0001, Naïve ****p <0.0001, time of interaction in the first session vs time of interaction in the second session) (B) Trial 2 (VH *p= 0.0499, time of interaction with novel rat2 vs time of interaction with novel rat3) ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; unpaired t test, Data expressed as Mean ± SEM).

4.4. Effect of Dorsal and Ventral Hippocampal Catecholamine Depletion on the Recognition Memory

The novel object recognition test was used for assessment of short-term recognition memory. There is no difference observed between DH, VH, Sham-V, Sham-D, and naïve groups in discrimination index and % novel object visits (Figure 4.7.A, 4.7.B). Also, no difference was observed in the distance travelled through the test session between the groups (Figure 4.7.C). These results might indicate that the DH and VH catecholamine depletions did not affect the short-term recognition memory in the study.

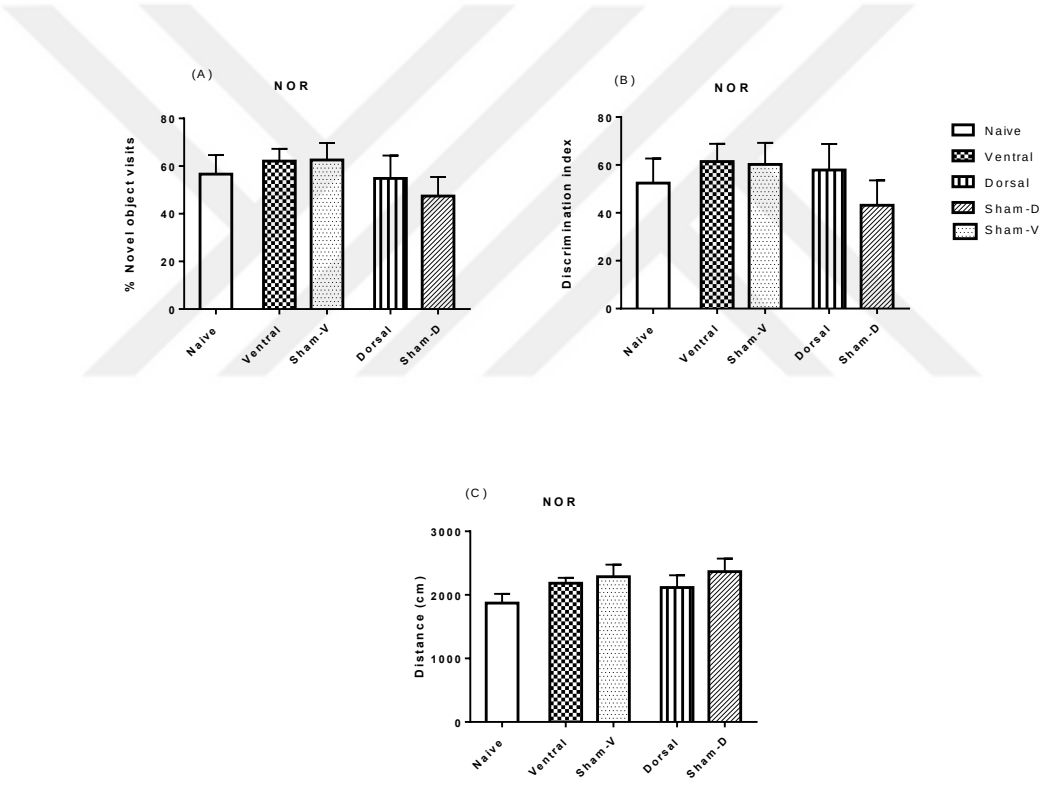


Figure 4.7. Effect of dorsal and ventral hippocampal catecholamine depletion on the recognition memory in NOR test. (A) percent of the number of visits to the novel object percentage (B) discrimination index (C) Distance travelled. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; one-way ANOVA, Multiple comparisons, Data expressed as Mean ± SEM).

4.5. Effect Of Dorsal and Ventral Hippocampal Catecholamine Depletion on Spatial Memory

To assess the short-term spatial memory function after the depletion of catecholamines, Y-maze test was used. There is no difference observed between DH, VH, Sham-V, Sham-D, and naïve groups in the % alternations (Figure 4.8.A). Similarly, no difference observed in the % visit to previous, same and new arms between in each group (Figure 4.8.C). Also, global path value showed no differences between the groups (Figure 4.8.B). This could indicate that the DH and VH catecholamine depletion did not affect the short-term spatial memory function in the study.

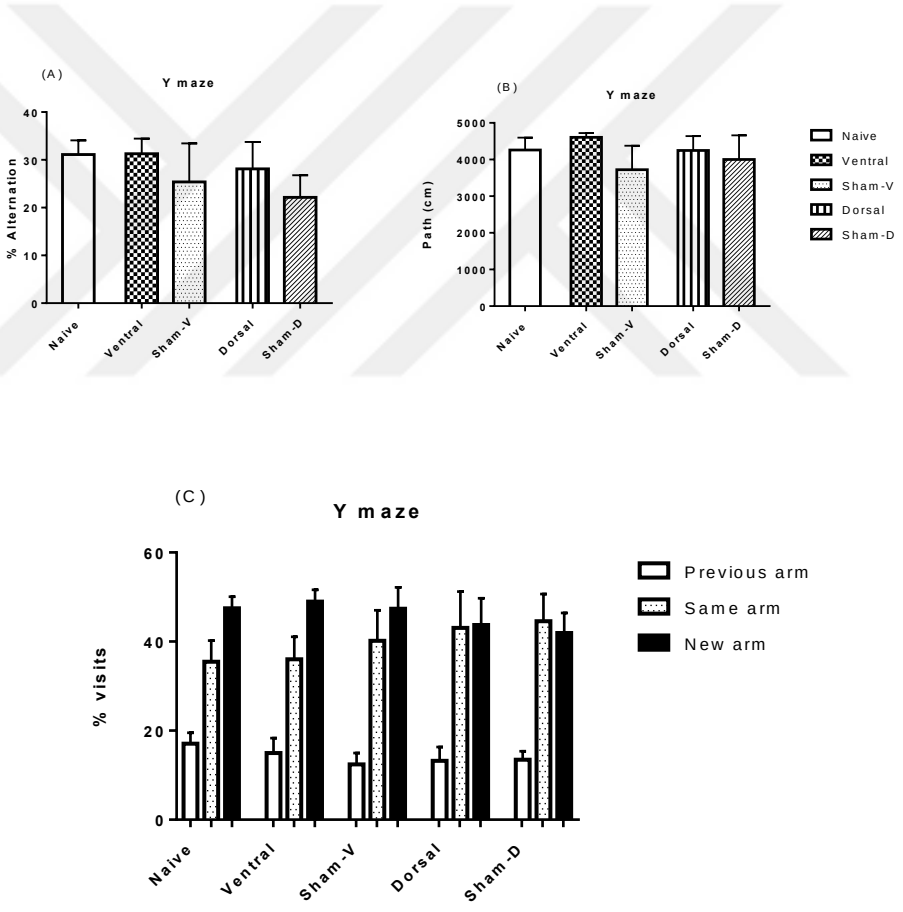


Figure 4.8. Effect of dorsal and ventral hippocampal catecholamine depletion on Spatial memory in Y-maze. (A) % Alternation (B) Global path traveled (C) %visits to previous, same, and new arms. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ =

8, n_{DH} = 10, n_{VH} = 10; one-way ANOVA, Multiple comparisons, Data expressed as Mean ± SEM).

4.6. Effect Of Dorsal and Ventral Hippocampal Catecholamine Depletion on Anxiety Behaviour

To measure anxiety-like behaviour, all the experimental groups were assessed for elevated plus maze test. There is no difference observed between the DH, VH Sham-V, Sham-D, and naïve groups in % time spent in the open arms nor the number of visits to open arms (Figure 4.9). This could indicate that the DH and VH catecholamine depletion did not affect anxiety-like behavior in our study.

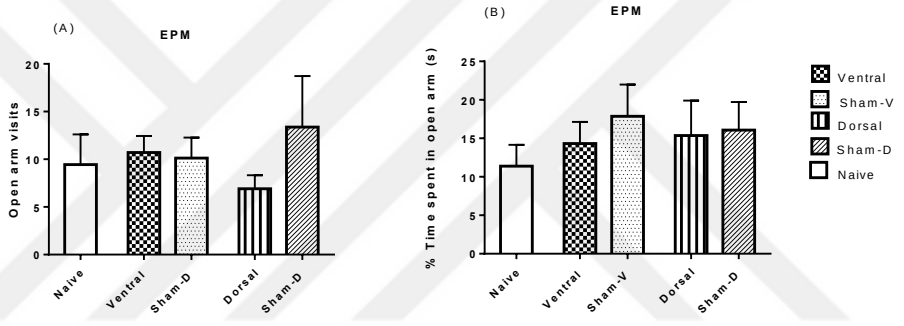


Figure 4.9. Effect of dorsal and ventral hippocampal catecholamine depletions on anxiety like behavior in EPM. (A) Open arm visits (B) % time spent in the open arms. ($n_{naïve}$=8, $n_{sham\text{-}D}$ = 8, $n_{sham\text{-}V}$ = 8, n_{DH} = 10, n_{VH} = 10; one-way ANOVA, Multiple comparisons, Data expressed as Mean ± SEM).

4.7. Effect Of Dorsal and Ventral Hippocampal Catecholamine Depletion on Anhedonia

Sucrose preference test was conducted to evaluate the animals for any symptom of anhedonia which is one of the core symptoms for depression. There is no difference observed between the DH, VH Sham-V, Sham-D, and naïve groups in percent of sucrose preference (Figure 4.10). DH and VH catecholamine depletions show the lack of effect on anhedonia.

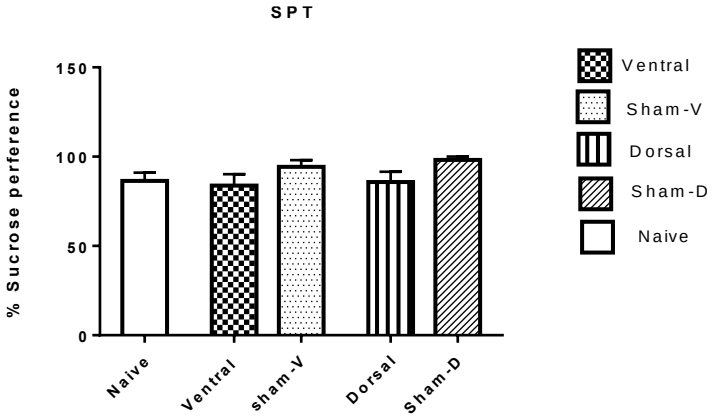
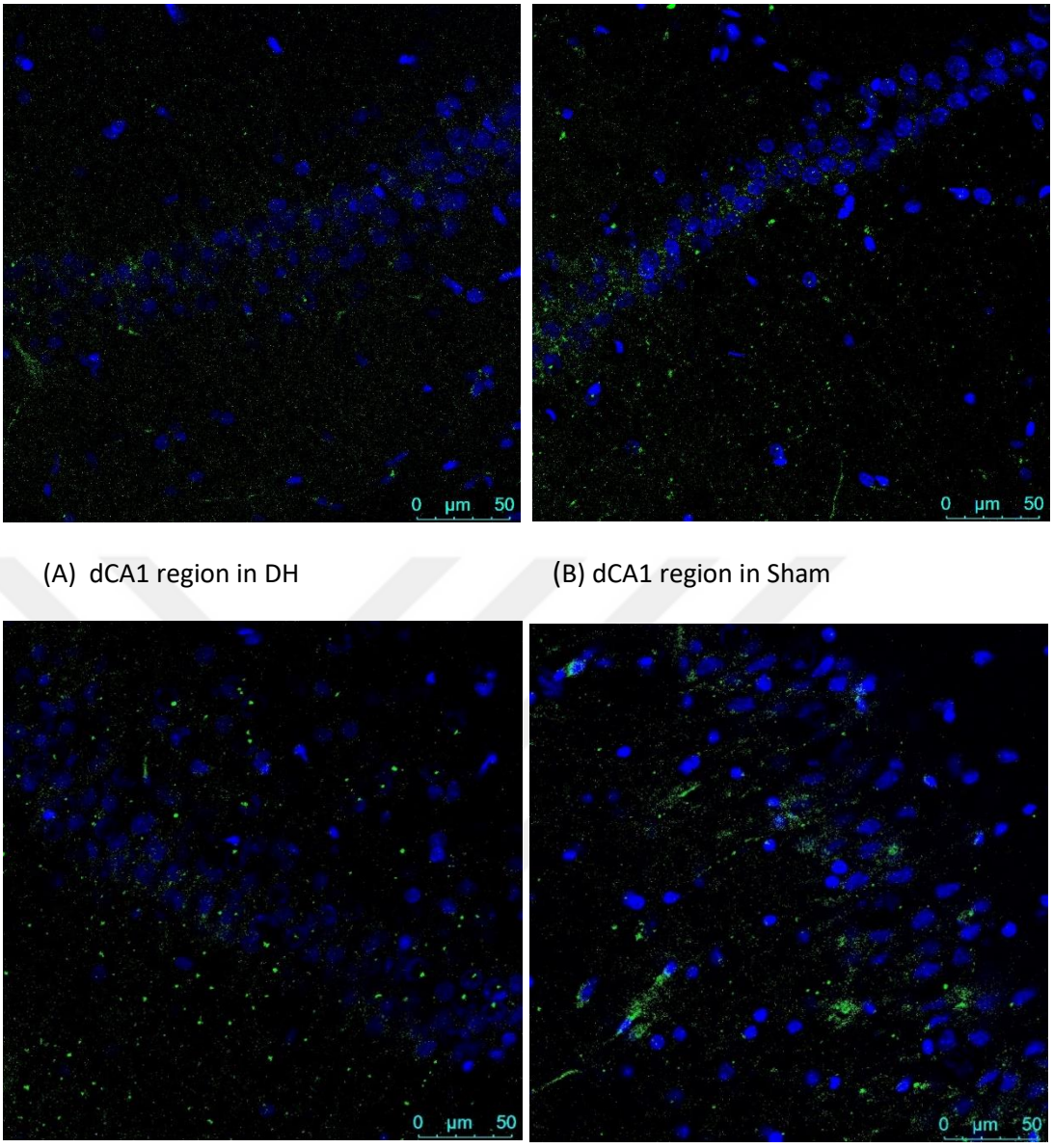


Figure 4.10. Effect of dorsal and ventral hippocampal catecholamine depletion on % sucrose preference. ($n_{naïve}$=8, n_{sham-D} = 8, n_{sham-V} = 8, n_{DH} = 10, n_{VH} = 10; one-way ANOVA, Multiple comparisons, Data expressed as Mean ± SEM).

4.8. Immunofluorescence Staining

A demonstrative staining for Hippocampal CA1 region has been performed using immunofluorescence staining. The initial representative images show some reduction of tyrosine hydroxylase signal, the known marker for catecholaminergic terminals in both dorsal CA1 (dCA1) in DH and in the ventral CA1 (vCA1) in VH groups compared to Sham-D and Sham-V groups respectively (Figure 4.10). This might be due to the depletion of catecholamine in these regions after 6-OHDA injection.



(A) dCA1 region in DH (B) dCA1 region in Sham

(C) vCA1 region in VH (D) vCA1 region in sham

Figure 4.11. Representative immunofluorescence staining of the dorsal hippocampal CA1 region (dCA1). The green color represents tyrosine hydroxylase positive (TH+) terminals, while blue color represents Hoechst stain of the nucleus. Upper panel shows the animals with dorsal hippocampus 6-OHDA injection (A) and sham injection (B), while the lower panel shows the animals with ventral hippocampus 6-OHDA injection (C) and sham injection (D); Scale Bar:50µm, 40X.

5. DISCUSSION

Pathological involvement of hippocampus has been described in the process of many neurodegenerative and neuropsychiatric disorders where cognitive decline including deficits in sociability and social memory have been reported (1, 2). Even though the role of the hippocampus in processing and storing different kinds of memories has been studied for a long time, not much is known about the neural circuit and type of hippocampal neurons that control social memory (3).

In our study we tried to understand the role of catecholaminergic innervations in dorsal and ventral parts of hippocampus in the social memory processing through depletion of catecholamines from the neuronal terminals using the 6-OHDA. Then, we combined both behavioral tests and tissue analysis to understand the mechanism of associated behavioral deficit that was observed in the animals.

5.1. Dorsal and Ventral Hippocampal Catecholamine Depletion due to 6-OHDA

In our study, we used a neurotoxin 6-OHDA to deplete the catecholamines from catecholaminergic terminals in either dorsal or ventral hippocampus. 6-OHDA acts through producing free radicals leading to oxidative stress after being taken up by the catecholaminergic neuron terminals leading to their degeneration that causes the depletion of catecholamines. Also, additional mechanism of actions has been described in several studies such as binding reversibly to mitochondrial respiratory chain complexes I and IV, causing impairment in energy metabolism (50). It also leads to concomitant activation of autophagy and apoptosis, beside neuroinflammation (51, 52).

6-OHDA is a neurotoxin that is not selective for one type of catecholamines, so we expected a depletion of dopaminergic, noradrenergic, and adrenergic terminals in the hippocampus (43, 44). The 6-OHDA depletion of the catecholamines have been observed within 3 hours for dopamine and throughout 72 hours for other catecholamines in neostriatal 6-OHDA-lesioned rat(43). Although the depletion occurs in the early days after neurotoxin injection, we preferred to give 10 days for

recovery before starting the behavioral tests. This is also to prevent any postoperative fatigue that could affect performance of the animals in the motor or behavioral tasks.

The recovery of the animals was evidenced by the absence of severe body weight loss (in some of the animals a slight increase in their body weight was observed) after the recovery period. Then, a set of behavioral tests were conducted for all experimental groups. All the tests were designed to be performed in a period of 28 days since previous research showed that throughout the course of four weeks, the reduction of striatal TH+ staining remained steady after 6-OHDA injection (53). While no such data was available for the hippocampal TH+ staining, we assumed the same time period and conducted all the behavioral tests throughout the four weeks to ensure consistent behavioral outcomes in our study.

It is important to mention that several studies used the noradrenergic reuptake inhibitor desipramine hydrochloride to increase the selectivity of the lesion for the dopaminergic neurons. However, such approach may be insufficient because other studies have shown that desipramine provides a moderate protection for noradrenergic neurons and also some loss of serotonergic and noradrenergic neurons has been observed (54, 55). In addition, many long-lasting side effects of desipramine have been observed in rats that could affect their performance in our behavioral tests, which led us not to choose such approach in our study (45).

In our study, we only focused on the depletion of catecholamines in hippocampal CA1 region as we observed that it is the densest TH+ containing region in hippocampus compared to other hippocampal regions. This will not rule out the depletion of the other neighboring hippocampal region such as CA2, CA3 and dentate gyrus which could need to be further studied in future research.

Our demonstrative immunofluorescence staining shows that there is a reduction of tyrosine hydroxylase signal, the known marker for catecholaminergic terminals, in the dorsal hippocampal CA1 region in the dorsal group and in the ventral hippocampal CA1 regions in the ventral group compared to the sham group. This

reduction in the signal could indicate the degeneration and depletion of catecholaminergic terminals in these regions.

Additionally, Hoechst staining for the nucleus did not show a substantial lesion in the ventral, dorsal, sham-D, and sham-V groups. This could indicate that the 6-OHDA did not cause a massive lesioning of all hippocampal regions, but selectively targeted the catecholaminergic terminals in CA1. Our findings are similar to the study done by Grau-Perales and his colleagues, which shows that injection of 6-OHDA into the hippocampus leads to loss of dopaminergic terminals in hippocampal CA1 and other hippocampal regions without causing nonspecific lesioning to all regions (44). Although their study has only focused on dopaminergic terminals, it did not rule out the depletions of other catecholamines in the region, which has been demonstrated by other studies (43, 45).

5.2. Dorsal and Ventral Hippocampal Catecholamine Depletion and Non-Cognitive Behaviors

It is always rational to combine non-cognitive tests in the assessment of cognitive function in rats which also affect their performance in behavioral experiments. Therefore, tests for locomotor activity, anxiety, and depression are usually included (56). Also, since we are evaluating social memory, assessing the olfactory function could be an important to perform before the social memory tests, as it is the main sensory modality in social memory of rodents (57).

5.2.1. Locomotor Activity

We started our behavioural tests by assessing the locomotor activity using open-field locomotor activity cages. We did not expect to observe any changes in locomotor activity following 6-OHDA injection into either DH or VH, since none of the previous studies were reported changes in locomotion in hippocampal 6-OHDA-leisoned animals (44, 45). Interestingly, we discovered that the ventral group has an increase in locomotor activity in terms of horizontal, ambulatory activity, and total distance traveled, but no similar hyperlocomotion was observed in the dorsal, Sham-

V, and Sham-D groups when compared to the naive. The effect of 6-OHDA lesion of the VH on locomotor activity is in agreement with several studies (58, 59). Zhung and his colleagues demonstrated that NMDA-lesion on the VH increased locomotor activity in open field test while no change was observed in the DH-lesioned group. Their study suggested that the bilateral lesions could activate the VH, which resulted in activation of the mesoaccumbal dopaminergic pathway leading to an increase in locomotor activity (58). In addition, the projection of the VH to the prefrontal cortex could also be included in the hyperlocomotion mechanism(58). Not only NMDA but also lesioning of the VH with ibotenic acid produced hyperlocomotion in neonatal rats in schizophrenia models (59). This could in part show that the different mechanisms may involve in VH to participate in the modulation of locomotor activity hence, the observation in our model is similar to the previously mentioned studies, although the mechanism of producing the effect might be different. Taking into consideration that 6-OHDA was found to induce “compensatory hyperinnervation” of the serotonergic neurons in different brain regions(60, 61), it seems possible that this mechanism could also be involved in the increase in locomotor activity . Another study showed that the injection of serotonin in the hippocampus led to increased activity of the animal tested in the open field (62). However, this interpretation should be made with caution because other studies show that 6-OHDA lesion could also reduce the serotonin level in the rat brain (63-65). This variation could indicate that the serotonergic hyperinnervation could be dependent on both dose and the site susceptibility to 6-OHDA, so further characterization is needed to understand these variations.

In a study that intracerebroventricular 6-OHDA injection of mice model of the attention deficit hyperactivity disorder (ADHD) showed an increase in locomotor activity (66). Our study could suggest that the VH could be a potential target that causes this hyper-locomotor activity in the 6-OHDA animal model of ADHD. One implication of our data is that it could help to understand the mechanism of the increased locomotor activity in ADHD.

Another significant aspect of our data is that it is relevant to positive symptoms of schizophrenia, which are produced in animal models by injection of ibotenic acid to the VH, so further validation of our model for possible use in schizophrenia research would be recommended (67, 68).

The dorsal group did not show any increase in locomotor activity, agreeing with one study reporting that the DH may lack the role in the modulation of locomotor activity (58). However, although there are studies that used 6-OHDA injections into hippocampus, there are no results reported regarding locomotor activity in their studies(44, 45). Our results suggest that the effect of 6-OHDA hippocampal lesion on locomotor activity are different between the dorsal and ventral hippocampus. This could be due to their differences in the type of modulation and connectivity to the other brain regions that control the movement.

5.2.2. Olfaction

Olfaction has been shown to play the most important role as sensory cue for social memory in rats, unlike humans whom the visual and auditory cues are the main sensory modality (69). Lesion of the hippocampus has not been recorded to interrupt olfactory discrimination or olfaction in rats (34, 70). Similarly, in our study, the depletion of catecholamines in either dorsal or ventral hippocampus did not affect the ability of rats to find the pellet in the BFST. Thus, our result excluded any false negative interpretation of social memory impairment due to impaired olfaction rather than hippocampal catecholamine depletion.

5.2.3. Dorsal and Ventral Hippocampal Catecholamine Depletion Did Not Affect Anxiety nor Depression Like Behavior

In EPM none of our groups showed a difference in the time spent in open arms which could indicate that the depletion of catecholamines in both dorsal and ventral hippocampus did not affect the anxiety like behavior. It has been shown that the ventral hippocampus is responsible for fear and anxiety behaviour (71). Disruption of the VH has been shown to produce an anxiolytic-like effect on rats while lesioning

the DH did not have any effect on anxiety (27). Therefore, we were expecting to see an anxiolytic-like effect after 6-OHDA injection into the VH. Interestingly, no effect was observed in anxiety-like behavior in VH group, which could be attributed to a lack of role of catecholamines in the type of anxiety-like behaviour in EPM, as in one study only showed a deficit in anxiety-like behaviour in specific anxiety tasks (27). Furthermore, ibotenic acid lesion of the VH in C57BL/6 J male but not female mice produce an anxiolytic-like effect in the EPM task, indicating a sex difference in VH modulation of anxiety-like behaviour (72). As we used female rats in our study, this could explain why we did not see any effect of the 6-OHDA on anxiety-like behaviour.

It is worth noting that a 6-OHDA lesion in the amygdala causes an increase in anxiety-like behavior, suggesting that the role of catecholamines may also be site-specific (73). The role of catecholamines in anxiety has been debated for a long time since (Dbh-/-) knockout mice that lack the dopamine beta-hydroxylase enzyme, which is responsible for noradrenaline synthesis, did not show altered anxiety behavior in EPM (74). Similarly, our findings could support this observation. Whether the role of catecholamines in anxiety is sex, site, or task dependent, extended studies are needed to include all these factors to understand the exact role of catecholamines in anxiety-like behavior.

We used the SPT to measure anhedonia as a parameter to investigate whether dorsal or ventral hippocampus catecholamine depletion could induce depressive-like state in the group. Although low level of hippocampal dopamine has been shown to induce depressive-like behavior in chronic restraint stress rat model of depression (75), We still did not observe any difference between the groups in percent of sucrose preference. This could be due to a minor depletion that occurred with 6-OHDA-lesionig or low sensitivity of the behavioral test that we used to assess the depressive-like behaviors.

5.3. Dorsal and Ventral Hippocampal Catecholamine Depletion and Cognitive Behavior

We studied the effect of catecholamine depletion in the dorsal and ventral hippocampus on different types of memory functions, including social, spatial, and recognition memory, in addition to sociability.

5.3.1. Dorsal and Ventral Hippocampal Catecholamine Depletion Affect Sociability and Social Memory

For the assessment of social memory, we used two paradigms: 3-CSMT for short-term social memory and 2-TDIT for long-term social memory. The rats have an innate preference to spend more time interacting with novel objects or rat rather than familiar ones(76, 77), thus, in the 3-CSMT, the time spent in the chamber has been taken as a measure to show the interest of the animals. Therefore, a typical rat should spend less time interacting with a familiar rat in the second exposure compared to the first exposure. This reduction was considered cognition or memory formation for this individual(77). The number of visits to each chamber could be used as a parameter to measure the interest. However, this approach did not always reflect social preference and it is better to be taken as a general indicator for normal locomotor activity (78).

Since DH regions have been involved in social memory (10, 34). As we expected, bilateral depletion of catecholamine in the DH impaired the ability of the animal to recognize familiar from novel juveniles in both 3-CSMT and 2-TDIT. These results agree with a study done by Stevenson and Caldwell, which shows that excitotoxic NMDA lesion of the DH CA2 region impaired social recognition memory of mice in two-trial social discrimination and an 11-trial habituation/dishabituation social recognition test (34).

Our demonstrative staining for the dorsal group shows that there is a reduction in tyrosine hydroxylase signal in the dorsal CA1 hippocampal region compared to the sham group. However, our findings could still provide some evidence for the importance of catecholaminergic terminals in the dorsal

hippocampal CA1 region in social memory processing. As we observed in our study, the reduction of tyrosine hydroxylase in dorsal hippocampal CA1 and the impaired social memory could be supported by the study done by Zinna and her colleagues, where they have shown the involvement of D1/D5 dopaminergic and β-adrenergic receptors in dorsal hippocampal CA1 in the consolidation of social recognition memory (10).

The effect of depleting dorsal hippocampal catecholaminergic terminals on social memory is also similar to a report by Griffin and his colleagues. They have shown that depletion of catecholamines in the locus coeruleus, which is one of the main sources of catecholaminergic terminals to the DH, was sufficient to prevent the rats from discriminating between novel and familiar animals (42).

Regarding sociability, the depletion of DH catecholaminergic terminals did not affect the preference of the animals to spend time interacting with another rat rather than staying in the empty chamber. This might indicate a minor or lack of role of the catecholamines in the sociability of rats in DH.

Moving to the effect of depletion of VH catecholamines on sociability and memory, the depletion impaired the sociability and social memory in 3-CSMT. We assumed that lesioning the VH, a structure that is known to be involved in anxiety and fear, could promote prosocial rather than withdrawal behavior. Interestingly, the ventral group exhibits no preference between spending time in the empty chamber and in the rat chamber, which indicates low sociability. These results seem to be similar to the social deficit that has been seen in the schizophrenia animal model in which the VH-lesioned in neonatal rats (59).

Alternative explanation for the observed deficit in sociability could be due to the 6-OHDA lesion that could lead to a decrease in parvalbumin-expressing cells, which in one study suggested that it could be as a result of the secondary loss of dopaminergic terminals (79). The loss of dopaminergic terminals could mean the loss of afferent neurons to the nucleus that could cause a change in the expression of parvalbumin (79). The parvalbumin-positive neurons in the VH are involved in the normal sociability and social memory, and their loss could be involved in the

mechanism by which 6-OHDA caused the deficit in sociability and social memory (80, 81).

Our study could be the first to show that 6-OHDA injection into the VH could produce animal models with negative and positive symptoms of schizophrenia, as our model manifested the hyperlocomotion and social deficit behaviors which are similar to schizophrenia phenotypes.

Besides low sociability, the ventral group showed impaired social memory in the 3-CSMT in which the animal was unable to recognize familiar juvenile rat. As a study has recently demonstrated that the ventral hippocampal CA1 region as a storage site for social memory which supports our finding (36). Therefore, we are the first to report that catecholamines in the ventral hippocampal CA1 region participate in the neural circuit that controls and stores social memory.

The first trial in the 2-TDIT, the ventral group showed a reduction in interaction when a previously met juvenile rat was introduced for the second time. Typically, this reduction would be considered as memory formation, leading us to question whether this group recognizes this familiar juvenile, or it is a false positive result. To confirm this, we performed trial 2, where two different novel juvenile rats were introduced. Similarly, the animals showed a reduction in the interaction time. Therefore, these contradictory results could be explained in two ways: the first explanation is that the reduction observed in both trials was only due to the low sociability and not memory formation, and this was supported by the performance of this group in the sociability session of the 3-CSMT, the second explanation is that the ventral group was able to recognize and form memory for the familiar conspecific, but they failed to distinguish between two novel conspecifics. The last explanation has been observed in a study done by Marino and his colleagues were showed that dopamine beta-hydroxylase knockout (Dbh -/-) mice were able to recognize a single rat when exposed twice, while they failed to recognize a novel rat (74). One could suggest that there might be different pathways for social memory encoding for familiar and novel conspecific, and these pathways could be modulated through

different types of neurotransmitter and neurons. But further studies are needed to test the scientific validity of such hypothesis.

Other significant value of our data is that we found the injection of 6-OHDA into the VH leads to impaired sociability, social memory, and hyperlocomotion. This phenotype could be relevant to ASD models as many genetic models of ASD manifest similar phenotypes. Furthermore, some animal models of ASD exert some abnormalities in the ventral hippocampus, such as decreased in the number of immature neurons or some hyperactivity in VH to the medial prefrontal cortex projections, which supports our findings (18, 82, 83). It is also worth noting that the Pak2+/ mouse model of autism has been shown to have impaired long-term potentiation (LTP) in the hippocampal CA1 region (84). Taking into consideration that 6-OHDA has been shown to cause impairment of hippocampal LTP in the Parkinson's disease model, that is thought to be dopamine dependent impairment (85). In our model the social deficit could be caused by LTP impairment induced by 6-OHDA injection. Therefore, our model could possibly mimic the Pak2+/− model and manifest similar phenotypes through the same mechanism.

In summary, our findings highlight the importance of catecholaminergic terminals of the VH in sociability and social memory, which has not been studied before. The study could lead to the development of a potential animal model for neurodevelopmental disorders such as ASD and schizophrenia, and further studies are needed to test the predictive validity of this model for potential future use in drug discovery. Finally, these results provide important insights about how dorsal and ventral hippocampal catecholaminergic terminals are important in social memory processing and the VH has more significant role in modulating sociability and locomotor activity in rats.

5.3.2. Dorsal and Ventral Hippocampal Catecholamine Depletion Did Not Affect Recognition nor Spatial Memory

Since many studies indicate the importance of hippocampal catecholamines in modulating novelty and novel object recognition memory (86, 87), we expected to

observe impairment in NOR test, however, in our study the depletion of the dorsal and ventral hippocampal catecholamines did not induce any impairment in recognition memory. One possible explanation for this finding is that the 6-OHDA lesion that we made was not sufficient to induce any impairment in recognition memory, as some memory tasks require a large tissue size lesion to manifest the memory impairment (88).

It is worth mentioning that we only measured the short-term memory, and this did not rule out the possibility that the long-term recognition memory might be affected by the depletion of catecholamines, so further studies are needed to assess the long-term memory.

Regarding spatial memory, both dorsal and ventral hippocampal catecholamine depletions failed to impair spatial memory in the Y-maze task. Our finding is contradictory to many studies that show the dopamine in the DH is responsible for the spatial learning and memory processing (29, 88, 89). Hence, lesion to the dorsal and -in some studies- ventral hippocampus could produce impairment in spatial memory as well (29, 88, 89). This could be due to a minor lesion that is not enough to produce impairment or different brain regions that could work independently from hippocampus to modulate spatial memory.

6. CONCLUSION

This study aimed to investigate whether dorsal and ventral hippocampal catecholaminergic innervations are important in social memory of rats. Our findings indicate that 6-OHDA injection could reduce the tyrosine hydroxylase in the hippocampal CA1 region, which suggests the catecholaminergic depletion. Furthermore, the depletion of catecholaminergic terminals in either dorsal or ventral hippocampus was sufficient to impair social memory which was tested with 3-Chambers and 2-Trial direct interaction social memory tests, was not due to impaired olfaction or locomotion. This indicates that both dorsal and ventral hippocampal catecholaminergic innervations are essential for normal social memory processing. In addition, depletion of ventral hippocampal catecholamines but not the dorsal ones, produced hyperactivity-like state and a deficit in sociability, which would suggest involvement of VH in modulation of locomotor activity and sociability in rats. This variation in the function could possibly be due to the differences in their connections to other brain regions. Further findings show that depletion of the ventral and dorsal hippocampal catecholamines did not induce depressive- or anxiety-like behaviors and did not impair recognition or spatial memory.

Our findings highlighted the importance of ventral hippocampal CA1 catecholaminergic innervations in sociability and social memory, which could be explored more in diseases such as ASD and schizophrenia. Furthermore, depletion of ventral hippocampal catecholamine produced phenotypes that could be relevant to the ASD animal model, such as hyperlocomotion, impaired sociability and social memory. Therefore, our model could be of value for the screening of drugs for symptomatic treatment in ASD and schizophrenia.

Our study did not specifically target an individual catecholamine or a hippocampal subregion, it is unclear whether these neurotransmitters have overlapping or dissociative functions in social memory. Also, the lack of quantification of the depletion could leave some questions ongoing about the level of depletion that is needed to manifest the social memory impairment. However, our study could open the door for future research to study the exact mechanism of hippocampal

catecholamine in modulating sociability and social memory, and to potential use of our model in ASD and schizophrenia research.

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8. APPENDICES

Appendix 1. Ethics committee permission for the study

T.C.
HACETTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu

Sayı : 52338575-44

HAYVAN DENEYLERİ ETİK KURUL KARARI

TOPLANTI TARİHİ	:	25.01.2022 (SALI)
TOPLANTI SAYISI	:	2022/01
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ONAY BİTİŞ TARİHİ		25.01.2027
ARAŞTIRMA YÜRÜTÜCÜSÜ	:	Doç. Dr. Banu Cahide TEL
HAYVAN DENEYLERİNDE GÖREVLİ ARAŞTIRMACILAR	:	Ahmed Algali Sedahmed Musa (Yüksek Lisans Tezi), Doç. Dr. Banu Cahide TEL, Ecz. Hilal AKYEL, Burcu Nur AKGÜNER
DİĞER YARDIMCI ARAŞTIRMACILAR	:	Doç. Dr. Gül Yalçın ÇAKMAKLI, Prof. Dr. Bülent ELİBOL, Dr. Elif ÇINAR
ONAYLANAN HAYVAN TÜRÜ ve SAYISI	:	***66 Adet Wistar Sıçan (10-12 Haftalık)***

Üniversitemiz Eczacılık Fakültesi Farmakoloji Anabilim Dalı öğretim üyelerinden Doç. Dr. Banu Cahide TEL'in araştırma yürütücüsü olduğu 2022/09 kayıt numaralı ***"Sıçanların Sosyal Hafızasında Dorsal ve Ventral Hipokampal Katekolaminerjik Nöronların Rolü ve Önemi (The Role and Importance of Dorsal and Ventral Hippocampal Catecholaminergic Neurons in the Social Memory of Rat)"*** isimli çalışma Hayvan Deneyleri Yerel Etik Kurulu Yönergesi'ne göre uygun bulunarak oy birliği ile onaylanmasına karar verilmiştir. Kurul Üyesi Doç. Dr. Banu Cahide TEL çıkar çatışması nedeniyle tartışma ve oylamaya katılmamıştır.

Araştırma yürütücüsü en geç, onay bitiş tarihinden sonraki 1 ay içerisinde proje sonuç raporunu Kurulumuza teslim etmekle yükümlüdür.

Prof. Dr. Sema ÇALIŞ
Etik Kurul Başkanı

Hacettepe Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu 06100 Sıhhiye-Ankara
Telefon: 0 (312) 305 1090-1082 • Faks: 0 (312) 310 0580
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TOPLANTI SAATİ	: 14.00
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THE ROLE AND IMPORTANCE OF DORSAL AND VENTRAL HIPPOCAMPAL CATECHOLAMINERGIC INNERVATIONS IN SOCIAL MEMORY OF RATS

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9. RESUME