ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DOPAMINERGIC MODULATION OF SPATIAL WORKING MEMORY IN MALE RATS: PRE- EXPERIENCE AND TASK DEPENDENT ROLES OF DOPAMINE D_1- and D_2-LIKE RECEPTORS

BY

MEKITE BEZU BEDANE

JUNE 2018

ADDIS ABABA, ETHIOPIA

Dopaminergic Modulation of Spatial Working Memory in Male Rats: Pre-Experience and Task Dependent Roles of Dopamine D_1- and D2-Like Receptors.

Mekite Bezu Bedane

A dissertation Submitted to Department of Pharmacology and Clinical Pharmacy, School of pharmacy, College of health Sciences, Addis Ababa University

Presented in Partial Fulfillment for the Requirements of the Degree of Doctor of Philosophy in Pharmacology.

Addis Ababa University

Addis Ababa, Ethiopia

June 2018.

.

This dissertation is based on the following papers.

1. **Repeated application of Modafinil and Levodopa reveals a drug-independent precise timing of spatial working memory modulation.**
    - **Behavioural Brain Research. (June 2016), 312, pp: 9-13.**

2. **Spatial working memory in male rats: pre-experience and task dependent roles of dopamine D1- and D2-like receptors.**
    - **Frontiers in Behavioral Neuroscience. (October 2017.) Volume 11 (196), pp: 1-8.**

ABSTRACT

Dopaminergic Modulation of Spatial Working Memory in Male Rats: Pre-Experience and Task Dependent Roles of Dopamine D1- and D2-Like Receptors.

Mekite Bezu,

Addis Ababa Univrsity, 2018

The dopaminergic system is known to be involved in working memory processed by several brain regions like prefrontal cortex (PFC), hippocampus and striatum. Previous studies have focused on assessing acute effects of drugs on dopamine's contribution to working memory in trained animals. Thus, little is known about effect of repeated application of dopamine targeting drugs throughout training. The present study aimed to determine effects of repeated administration of dopaminergic drugs and to delineate the role D1-like and D2-like receptors (D1R and D2R) subtypes play in spatial working memory modulation and the task -dependent differences in performance.

Spatial working memory performance in male Sprague-Dawley rats was assessed by repeated intraperitoneal (IP) application of levodopa (2 or 20 mg/kg) or modafinil (1or 10 mg/kg) through 6 days training or intracerebroventricular (ICV) application of a D1R (SKF81297) and D2R agonist (Sumanirole) and antagonist (SCH23390, Remoxipride) at low (1μg) and high (5μg) dose through 3 days of training. Working memory performance test was carried out using T-maze. The experiment was repeated in a water maze for the most effective ligand to test for the task-dependent differences in working memory modulation. In addition, cAMP level in the PFC was assayed for ligands tested in the T-maze.

Enhancement in spatial working memory performance was observed at day 3 after levodopa ($p < 0.007$) but not modafinil administration compared to vehicle-treated animals. Low dose modafinil treated groups performed better than high dose treated rats and the reverse was true for levodopa treated rats. In T-maze, the D1R agonist enhanced working memory performance across training at low dose ($p < 0.007$), while the high dose induced enhancement only at day1 compared to controls. On the other hand, D1R antagonist showed persistent enhancement of working memory across training at high dose ($p = 0.013$), whereas no statistical difference was observed in low dose. The D2R agonist at both doses was not effective, but low dose of the D2R antagonist enhanced working memory at day 2.

In water maze task, no significant difference was observed at both doses of the D1R agonist (as it was the most effective compared to controls in T-maze). However, behavioral performance of all groups tested in water maze task was different compared to T-maze trained rats. cAMP levels were not significantly different between D1R agonist and control groups. Higher levels were, however, obtained in D1R antagonist (low dose) and D2R agonist (high dose) treated rats.

The data collectively support the view that modulation of spatial working memory is optimized within a limited range of dopaminergic transmission. However, it suggests that these ranges vary at different time points during spatial training and are also task dependent.

Key words. Dopamine, Working memory, T-maze, dopamine receptor D1R, dopamine receptor D2R, water maze.

ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere gratitude to my advisor, Professor. Ephrem Engidawork, for the knowledge and inspiration that he has granted me during my PhD study. I am also indebted to him for his patience, motivation, and immense knowledge. His help in finding research facilities and in sending me to abroad University, his guidance and support during the development of this work and writing of this thesis has been very much appreciated. Without his precious support it would not be possible to conduct this research.

Besides my advisor, during my time at Medical University of Vienna, there have been other mentors that have helped me. Hence my sincere thank also goes to Professor Gert Lubec, who provided me an opportunity to join his lab team, and who gave access to the laboratory facilities. I am also grateful to him for his encouragement and valuable help.

I would also like to express my gratitude to Professor Volker Korz for being always ready with guidance in the lab, and for introducing me to the techniques of behavioral studies and allowing me to learn other related lab techniques. I am grateful to have been able to work with and learn from him.

I would also like to thank all the students and labmates that I have had the privilege of working with, especially Daniel, Jovana Maliković and Martina Kristofova. I really appreciate all the time and effort that you all put into these experiments.

I would also like to thank the Pharmacology and Clinical pharmacy Department of school of Pharmacy for their help and support throughout my study.

I am grateful to my incredibly supportive parents. I cannot express how much their support means to me; it is the foundation to all of my successes.

Finally, I must express my very profound gratitude to my family (my beloved wife Netsi and my sweet children Nani and Miki) for providing me with unfailing support and continuous encouragement throughout my years of study and for their patience and understanding also during those times when this study made me less available to them. This accomplishment would not have been possible without them. Thank you.

TABLE OF CONTENTS

List of Abbreviations

6-OHDA	6- hydroxydopamine
ACg	Anterior cingulate
CA	Cornu ammonis
D1R	D1-like receptors
D2R	D2-like receptors
DAT	Delayed alternation task
DG	Dentate gyrus
DLPFC	Dorsolateral prefrontal cortex
DMTS	Delayed match-to-sample
DNMTS	Delayed non-match-to-sample
DRT	Delayed-response task
EC	Entorhinal cortex
Fmri	Functional magnetic resonance imaging
ICV	Intracerebroventiricular
IL	Infralimbic
IP	Intraperitoneal
LEC	Lateral entorhinal cortex
LO	Lateral orbital
MEC	Medial entorhinal cortex
MO	Medial orbital
mPFC	Medial prefrontal cortex
PET	Positron emission tomography

PFC	Prefrontal cortex
PrC	Precentral
PrL	Prelimbic
VLO	Ventro lateral orbital
VO	Ventral orbital
WME	Working memory error
WMI	Working memory index

List of Figures

List ***Page.***

1. Introduction

1.1 The concept of working memory

Spatial working memory is a highly dynamic process of short-time encoding of spatial information (Dudchenko, 2004) to adjust subsequent behavior. Working memory differs from short-term memory and long-term memory due to its essentially "executive" function, since it basically stores information for a very short period of time (seconds to minutes) in order to compare it with previous records, and, based upon this, decide which behavior to express. Short-term memory is a limited capacity store that, for example, can be assessed with a digit span task. Most people can hold between five and nine items in short-term memory (Miller, 1956). In Short-memory, information is kept for a limited amount of time until it fades or is transferred to a permanent state. In contrast, working memory is a capacity limited store that is less transient and more durable than short-term memory (Baddley, 1986). Information that is stored in long-term memory may last a lifetime. Many theorists view working memory as the subset of knowledge in long-term memory that is currently activated (Baddeley and Logie, 1999, Cowan, 1999; Oberauer, 2002).

Working memory is essential for facilitating complex behaviors and is considered critical for performing a variety of cognitive functions, including reasoning, problem solving, language understanding, thinking, planning and decision-making (Baddeley, 1986, 1992). Intact working memory enables to initiate appropriate behaviors based on the information being held on-line in real time. Individuals with impairments in working memory may have difficulties with a wide range of cognitive functions including language comprehension, problem-solving and planning,

which then could lead to reduced social functioning. The anatomical basis of working memory has been extensively studied and researchers found an implication of many structures most importantly the prefrontal cortex, thalamus, stratium, medial-temporal region like hippocampus and the parietal cortex.

Research in rodents commonly uses a broader definition of working memory, that refers to *"a* collection of processes that include the temporary storage of information, as well as executive functions that mediate the manipulation and retrieval of trial-unique information to guide action after both short (seconds) and longer (minutes to hours) delays" (Floresco and Phillips, 2001).

In rats, a similar distinction in memory is made between working memory and Reference memory. Reference memory refers to the long-term storage of information that remains constant over time and that is gradually acquired over many training sessions, whereas working memory is a specific form of short term memory that refers to the ability to retain information within a single trial (Olton, 1979). Importantly, this definition includes a much larger range of delays (seconds to many hours) compared to what is typically used in humans and non-human primates (seconds).

1.2 Models of working memory

Several models of working memory have been proposed and the most influential ones are reviewed below.

1.2.1 Baddeley's model of working memory

Baddeley and Hitch (1974) originally proposed that working memory consists of three main components: The central executive and two subsidiary slave systems: the 'phonological loop' and the 'visuospatial sketchpad'. Baddeley expanded upon this model in his seminal book (Baddeley,

1986) and has further modified his model (Baddeley, 2000) by adding a fourth component called the "episodic buffer".

The phonological loop is assumed to hold verbal information by using a temporary store and an articulatory rehearsal system. It is a system for performing speech perception and language comprehension and includes a mechanism for temporarily maintaining speech-based information by subvocal rehearsal (Baddeley, 2003). Meaningful phonological information may aid short-term recall by activating relevant information from long term memory (Baddeley, 1992; 2003).

Visuospatial sketchpad is a system for processing visuo spatial information as well as information that cannot be expressed by language and includes a mechanism for temporarily maintaining information as visuo-spatial images by a rehearsal system. It records visually presented sensory data and believed to be fractionated into separate visual and spatial components (Baddeley & Logie, 1999).

An episodic buffer is assumed to be a limited capacity storage system of about four episodes or chunks (Baddeley etal; 2010). It is capable of integrating information from a variety of sources. It serves as a passive store that combines visual and auditory information into multidimensional representations or chunks. It is controlled by the central executive, which is able to retrieve information from the store in the form of conscious awareness, or reflect on that information and, where necessary, manipulate and modify it (Baddeley, 2000; 2002). The buffer is episodic in the sense that it holds episodes whereby information is integrated across space and potentially extended across time.

The central executive controls attention and is responsible for regulating and coordinating the slave systems. Whenever an individual must process and store information, the central executive is involved. It simultaneously selects strategies, controls attention, and integrates information from a variety of sources on separate tasks. In regards to attention, it can attend selectively to specific information while inhibiting irrelevant information, which can help monitor many complex cognitive processes.

The model of working memory proposed by Baddeley is a conceptual model, not a structure based model. Therefore, none of four working memory components correspond to any particular brain structure, although the function of the central executive is thought to be related to the function of the dorsolateral prefrontal cortex (DLPFC) (Baddeley, 1986). It is clear that this model of working memory cannot be adapted to animals because the linguistic information treated by the phonological loop cannot be processed in non humans (Nadel and Hardt, 2010).

1.2.2 Cowan's model of working memory (Embedded Processes Theory)

Cowan defined working memory as "cognitive processes that are maintained in an unusually accessible state" (Cowan 1999, 2005). His theory involves a limited-capacity attentional focus that operates across areas of activated long term memory. Cowan (1999) suggested that working memory information comes from hierarchically arranged processes. The currently activated features comprise a subset of long-term memory, and the current focus of attention is in turn a subset of this activated memory. The capacity according to this model is four chunks or episodes, each of which may contain more than a single item (Cowan 2005). Dehn (2008) postulated that this model has some similarities to Baddeley's model in that the amount of information capable of

being held is dependent on the complexity of the information. Activation is time limited whereby activation of information within working memory will decay unless reactivated through rehearsal.

1.2.3 Oberauer's model of working memory

This is similar to Cowan's Embedded processes theory in that memory items may exist in varying states of accessibility (Oberauer 2002). Recently processed items have the most activated representations and are immediately accessible. However, it disagrees with Embedded processes theory in terms of the capacity of this component. According to Oberauer, only one item or chunk may be focused on at any given time – not four.

1.2.4 Executive attention model (Individual Difference–Based Theories)

According to this model individual differences on measures of working memory capacity primarily reflect differences in capability for controlled processing (Engle, 2001) and, thus, will be reflected only in situations that either encourage or demand controlled attention. It is the ability to control attention in order to manage and recall relevant information that determines working memory capacity rather than short-term span. Working memory capacity reflects the ability to apply activation to memory representations, to either bring them into focus or maintain them in focus, particularly in the face of interference or distraction (Engle et al. 1999; Engle and Kane, 2004). This model also emphasizes the role of working memory in retrieving and actively maintaining information from long term memory (Kane et al., 2001). This model is different, yet not entirely inconsistent, with Baddeley's model, which includes the central executive that controls attention (Dehn, 2008). The difference between the two models is in regards to what determines working memory capacity.

1.3. Brain regions involved in working memory

Working memory depends on a variety of interconnected brain regions, but most of the research supports the main involvement of the PFC and the hippocampus (Yoon et al., 2008).

1.3.1. Prefrontal cortex

The PFC is a collection of distinct cortical areas located anterior to the frontal eye fields in the frontal lobe. It is most prominent in primates, especially humans than small animals, and is critical for higher-order cognitive processes and emotional regulation. This anatomical difference is thought to account for some of the pronounced differences in cognitive ability that are observed between species (Squire, et al. 2003).

The primate PFC is organized into several sub regions but can be broadly separated into a dorsolateral division that is involved in cognitive functions such as executive control, attention and working memory, and a ventromedial (or orbitomedial) division more involved in emotional and motivational regulation (Fuster, 2015). In rats, the anatomical definition of the PFC is not as clear as in monkeys (Uylings et al., 2003). A classical definition of the PFC in primates is the existence of granular layer IV, therefore, the PFC has been referred to as the ‘‘frontal granular cortex’’, but there is no such area in the rat frontal cortex.

In rodents the PFC can be defined, like in primates, as the cortical region receiving its main thalamic input from the mediodorsal thalamus (Uylings and van Eden, 1990). Researches using non-human animals have suggested that the medial prefrontal cortex (mPFC) in rodents may be homologous to the DLPFC in humans (Uylings et al. 2003; Kellendonk et al. 2006). The rodent PFC is typically divided into medial, lateral and ventral subdivisions, each of which in turn

consists of several sub-regions (Figure 1). A medial division can be sub-divided into a dorsal sub-region that includes precentral (PrC) and anterior cingulate (ACg) cortices and a ventral component that includes the prelimbic (PrL), infralimbic (IL) and medial orbital (MO) cortices. A lateral region includes the dorsal and ventral agranular insular (AID, AIV) and lateral orbital (LO) cortices. A ventral region encompasses the ventral orbital (VO) and ventro lateral orbital (VLO) cortices (Uylings and van Eden, 1990).

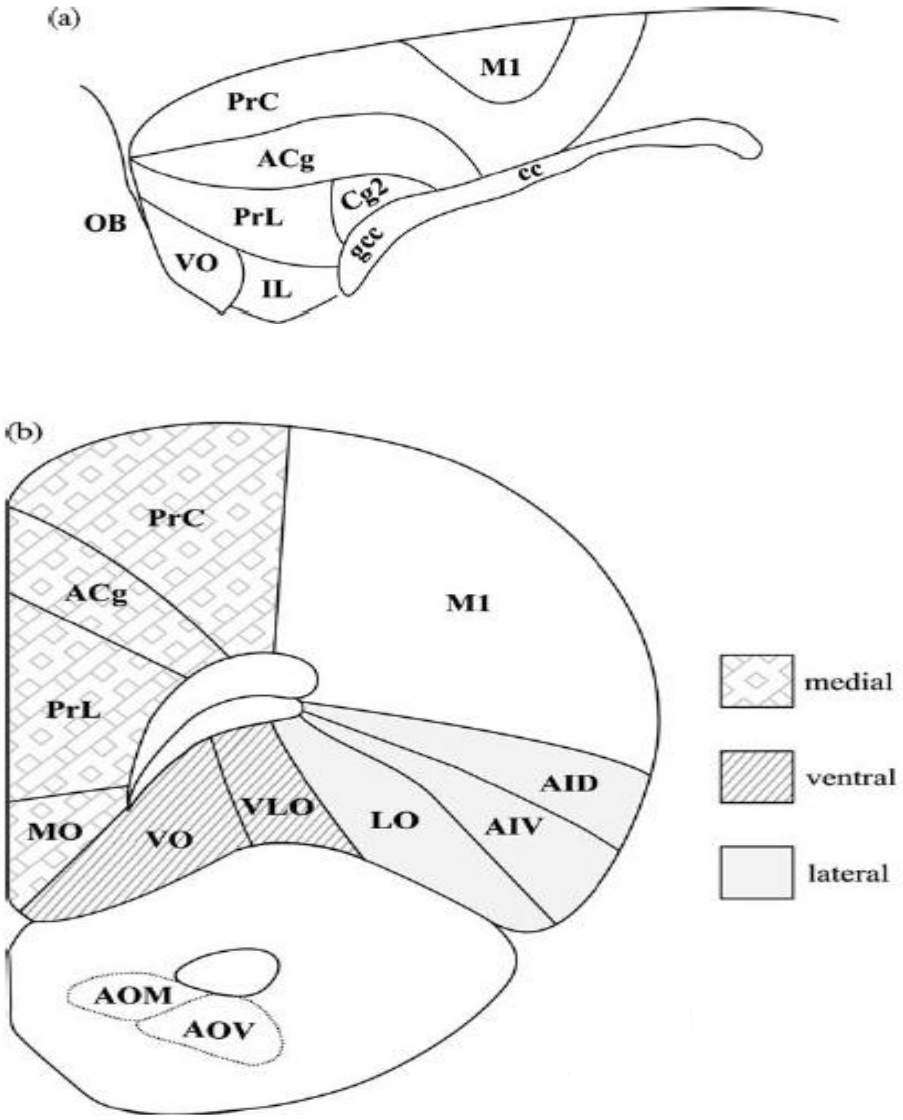


Figure 1: Structural organization of rat prefrontalcortex. Adapted from Dally etal (2004)

(a) Lateral view, 0.9 mm from the midline. (b) Unilateral coronal section, approximately 3.5 mm forward of bregma. Abbreviations: ACg, anterior cingulate cortex; AID, dorsal agranular insular cortex; AIV, ventral agranular insular cortex; AOM, medial anterior olfactory nucleus; AOV, ventral anterior olfactory nucleus; cc, corpus callosum; Cg2, cingulate cortex area 2; gcc, genu of corpus callosum; IL, infralimbic cortex; LO, lateral orbital cortex; M1, primary motor area; MO, medial orbital cortex; OB, olfactory bulb; PrL, prelimbic cortex; PrC, precentral cortex; VLO, ventrolateral orbital cortex; VO, ventral orbital cortex.

Many lesions, electrophysiological and imaging studies have highlighted the crucial role of the PFC in working memory (Funahashi and Kubota 1994). The importance of frontal lobe for performance on a spatial delayed alternation task in monkeys was first described by lesion studies (Jacobsen 1935). A number of subsequent lesion studies in monkeys within the PFC indicated that the DLPFC is the most critical region for visuospatial delay task performance (Goldman and Rosvold, 1970; Funahashi et al., 1993; Petrides 1996).

Neurophysiological unit recordings from the DLPFC often show persistent and sustained levels of neuronal firing during the retention interval of delayed response (Kubota and Niki, 1971). Furthermore, anatomically also, the lateral PFC is well interconnected with various sensory association and higher motor cortices. It may be mainly concerned with interaction with the external world, such as perception and recognition of external stimuli as well as planning and execution of motor actions. Such compelling data established a strong link implicating the DLPFC as a crucial node supporting working memory. Brain-imaging studies, using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), have also implicated the human PFC in working memory (Cohen et al. 1997; Courtney et al., 1998).

Deficit of visuospatial memory in rats was first demonstrated by lesioning mPFC of frontal lobe and using a delayed alternation task (DAT) in a T-maze (Kolb et al., 1974). Since then, a number of studies, specifically lesions to the prelimbic cortex have been shown to broadly impair working memory performance (Cai and Arnsten 1997, Heidbreder and Groenewegen 2003; Di Pietro et al. 2004; Gisquet-Verrier and Delatour 2006, Euston et al., 2012).

A sustained delay in activity similar to that found in monkeys has been found in the dorsal mPFC of rats performing the delayed response task. Furthermore, anatomical connections and functional roles of different mPFC subdivisions has shown that the DLPFC in monkeys and the dorsal mPFC in rats have much in common, such as that they are both the major targets of parieto-frontal projections (Hoover and Vertes, 2007; Churchwell et al., 2010; Yang etal., 2014). Improvements in working memory by direct injections of a D1 agonist into the prelimbic cortex (Cai and Arnsten 1997) and impairments in working memory and attention-based tasks by D1 antagonists (Granon et al.,. 2000) would, also indeed, show that rodents possess a prefrontal cortical area that is homologous to the DLPFC in humans (Uylings et al., 2003; Kellendonk et al., 2006). Thus, several lines of evidence indicate that in both monkeys and rodents, the PFC plays a critical role in working memory. These similarities also suggest a functional comparability of the DLPFC in primates and the mPFC of rodents.

1.3.2. Hippocampus.

For a long time, the hippocampus was believed to have a limited role in working memory (Milner et al., 1998) while others suggest that the hippocampus plays a significant role in working memory (Floresco et al., 1997, Lee and Kesner, 2003, Saxe et al., 2007). The hippocampus, a structure

heavily involved in visuo-spatial information processing, recently gained more recognition as an anatomical site for information storage during spatial working memory tasks.

The rat hippocampal formation is an elongated, sea-horse shaped structure. Its long axis extends in a C–shaped manner from the midline of the brain near the septal nuclei over and behind the thalamus into the incipient temporal lobe (Witter and Amaral, 2004). The term Hippocampal complex includes both the hippocampal formation and the parahippocampal region (Witter and Amaral, 2004). The hippocampal formation consists of three zones: the subiculum, the dentate gyrus (DG) and the hippocampus proper composed of Ammon's horn (*cornu ammonis* or CA). The parahippocampal region includes the entorhinal cortex (EC), the perirhinal and the parahippocampal cortices (Nadel and Moscovitch, 1998).

The hippocampus consists of structurally dissimilar processing subfields that are interconnected serially as well as directly with the EC (Witter and Amaral, 2004). This arrangement suggests that individual subfields may sub serve discrete functions. The hippocampus consists of two c-shaped laminae that wrap around each other. The first of these c-shaped structures, the CA, extends from the parahippocampal gyrus, which is found on the medial surface of the temporal lobe. It is commonly further divided into subregions termed CA1 through CA4.

The CA1 area constitutes the primary output from the hippocampus to the neocortex. It appears that CA1 plays a role in allocentric spatial tasks because specific lesions to this subregion induce impairment in such tasks (Stubley- Weatherly et al., 1996, Kesner et al., 2002). The CA3 subfield of hippocampus seems to be critical for the retrieval, rather than the encoding of memory (Rolls and Kesner, 2006).

DG Wraps around the CAs and termed after its teeth-shaped appearance from a medial view (Kandel, 2013). DG Receives and processes the first projections from the EC to the hippocampus. Thus, this structure is in a key position to control the flow of information to the hippocampus. Selective lesions to the DG resulted in impairments similar to the effects caused by a complete hippocampal lesion (Okada and Okaichi, 2009). The DG has also been well placed in the role as an encoder of newly acquired spatial information (Lee and Kesner, 2004).

The internal circuitry in the hippocampus

The hippocampus receives the bulk of its cortical afferents from the EC in the parahippocampal gyrus. In the trisynaptic pathway, pyramidal cells send projections via the perforant path to granular cells in the DG. The mossy fiber axons of these cells in turn terminate in CA3, whose pyramidal neurons make synaptic contact with those of CA1, via the Schaffer collaterals. CA1, finally, projects back to the EC. A direct pathway also exists, consisting of projections from the EC directly to the CA1. Thus, information flows through the hippocampus in a largely serial and unidirectional fashion (Duvernoy, 2005). The hippocampus is well interconnected with the neocortex. The perirhinal and parahippocampal cortices, both part of the parahippocampal gyrus, receive input from uni- and polymodal association cortices, which they in turn project onto the hippocampus via the EC (Suzuki & Amaral, 1994). The hippocampus thus receives highly processed input from distributed brain regions.

A Study has shown that for short-term delays the hippocampus and the PFC might process working memory information in parallel, but as soon as the system detects a longer delay, hippocampal memory may become essential, demonstrating more persistence than the PFC (Lee

and Kesner, 2003). The dorsal hippocampus is thought to process spatial information whereas the ventral pole is associated with emotion-related processing (Deguchi et al., 2011).

1.3.3. Hippocampus- PFC interaction.

The interactions between the hippocampus and PFC have emerged from animal studies as playing a key role in various cognitive and behavioral functions (Harris and Gordon, 2015). The PFC and hippocampus may interact via their direct/ indirect anatomical connections (Laroche et al., 2000; Thierry et al., 2000). In both rodents and primates a monosynaptic direct interaction involves projections from the hippocampus to PFC originates almost exclusively in the ventral hippocampus and primarily target the mPFC. There are no projections to the mPFC from the dorsal hippocampus or DG (Hoover and Vertes, 2007). The fibers navigate ipsilateral in the PFC through the fimbria/fornix system before terminating in the IL, the PL PFC and anterior cingulate cortex (Hoover and Vertes, 2007). These neurons are glutamatergic projection (Thierry et al., 2000). In mouse, Rajasethupathy et al. (2015) have identified a monosynaptic projection from the PFC to the dorsal hippocampus. This projection originates in the AC subdivision of the mPFC and terminates in the CA1 and CA3 subfields of the dorsal hippocampus. Bidirectional interactions between the two structures could also be achieved via several indirect routes. Indirect multi-synaptic pathways from the hippocampus to mPFC include projections through the Nucleus accumbens (NACC) and ventral tegmental area (VTA), amygdala, EC, and midline thalamus: Among these the PFC-nucleus reuniens (NR)-Hippocampus Loop have been shown to be critically involved in higher cognitive function (Vertes, 2006; Cassel et al., 2013).

PFC-NR-Hippocampus Loop.

The NR of the thalamus, which is reciprocally connected to the dorsal and ventral hippocampus as well as the mPFC, receives mPFC innervation and transmits processed information to the hippocampus by monosynaptic projection (Varela et al., 2014). The ventral mPFC exclusively targets medial structures of the thalamus while dorsal mPFC distributes primarily to the intralaminar, ventral, and lateral thalamus. It has been shown that single NR neurons send collaterals to both the hippocampus and mPFC (Hoover and Vertes, 2012; Varela et al., 2014). This places the NR in a key position to relay information between the mPFC and hippocampus to coordinate their functions (Griffen et al., 2015). The lateral EC is also reciprocally connected with the PFC as well as the hippocampus (Moser et al., 2010).

Studies suggest that both hippocampus and PFC are critical for spatial working memory, as damage to either structure results in spatial working memory impairment (Wang and Cai 2006). This raises the possibility that the direct Hippocampus-PFC projection mediates activity critical to the successful performance of spatial working memory dependent behavior. Several studies in rodents demonstrate the role of communication between the hippocampus and mPFC in the radial arm maze and T-maze tests (Floresco et al., 1997, Wang and Cai 2006). In these tasks the experimenters used the so-called ‘‘disconnection lesions’’ or asymmetric lesion in which the hippocampus is lesioned (or inactivated) in one hemisphere and the PFC in the opposite hemisphere. Such a lesion prevents communication between the remaining hippocampus and PFC. In those studies impairments in spatial working memory performance were observed. Evidence from researches using the neuronal silencer archaerhodopsin by optogenetic methods (Spellman et al., 2015) further suggested that hippocampal-prefrontal interactions are evident in spatial working

memory tasks. In the task, silencing of hippocampal-prefrontal inputs of mice resulted in impaired spatial working memory performance. Indirect projections from the mPFC back to the Hippocampus are also involved in working memory (Hembrook et al., 2012). Thus, both direct and indirect connections between the hippocampus and mPFC contribute to the hippocampal-prefrontal interactions important for working memory processes as well as spatial navigation.

Electrophysiological studies have also shown that cellular activities in these two regions are intimately coordinated during working memory tasks (Ferino etal., 1987; Hyman et al., 2005). Studies in both animals and human subjects have revealed correlations in neural activity between brain regions that often change with various task demands (Harris and Gordon, 2015). During a spatial working memory task, hippocampal-PFC synchrony was highest as animals approached the decision point (Jones and Wilson, 2005). These results suggest that hippocampal- PFC interactions may play a more general role in decision-making based on spatial information (Yu and Frank, 2015). Disruptions in hippocampal-prefrontal interactions have been observed in psychiatric disease, most notably schizophrenia and have also been reported in animal models of the illness (Sigurdsson, 2015).

1.4. Dopamine and Working memory

1.4.1. Overview of the Dopaminergic system

With modern immunohistochemical techniques, it has been possible to map out in detail the location of dopaminergic neurons and their specific projections (Hokfelt et al., 1977). The major groups of dopaminergic neurons are classified as A8-A17 (Figure 2). These groups are functionally divided in to four main groups, each of which has activity with a unique set of physiological and psychological effects (Stahl, 2000). The mesencephalic or midbrain

dopaminergic neurons comprised of groups A8-A10, the diencephalic dopaminergic neurons comprised of groups A11-A15, dopaminergic neurons in the olfactory bulb (A16), and the dopaminergic neurons located in the retina (A17).

The mesencephalic, or midbrain, dopaminergic system is further sub-divided into three separate pathways, the nigrostriatal, mesolimbic, and mesocortical pathways, all of which originate from the A8-A10 cell groups. These dopaminergic neurons originate in several neighboring mid brain nuclei, being the substantia nigra parscompacta (SNc; A9) and ventral tegmental area (VTA; A10). The nigrostriatal dopaminergic pathway, consists of neurons whose cell bodies originates primarily from the A9 group of SNc and to a lesser degree the A10 neurons of the VTA and terminate in to the dorsal striatum structures including the caudate, putamen, and globus pallidus, and is important in the regulation and coordination of locomotor activity (Ungerstedt, 1976). Unlike the nigrostriatal pathway, majority of the neurons that make up the mesolimbic dopaminergic pathway originate from the A10 neurons of the VTA, with fewer neurons originating from the A8 and A9 groups, and project to the nucleus accumbens, amygdala, and olfactory tubercle. In addition to its role in the regulation of affect, emotion, and locomotor activity, the mesolimbic dopaminergic pathway has also been implicated in reward and pleasure, and is often referred to as the "reward pathway" of the brain.

The mesocortical dopaminergic pathway projects to various areas of the PFC, including the orbitofrontal, medial, dorso lateral and cingulated regions (Abi-Dargham and Moore, 2003). The mesocortical dopaminergic neurons appear to be important for social behavior, working memory, attention, and executive function (Bubser and Schmidt, 1990; Sawaguchi and Goldman-Rakic, 1994; Floresco and Magyar, 2006).

The diencephalic dopaminergic system is further classified as the diencephalospinal, incertohypothalamic, and tuberoinfundibular dopaminergic pathways. The diencephalospinal pathway sends projections to the spinal cord and, to a lesser degree, the dorsal raphe nucleus and has been shown to be involved in dopamine-mediated nociception, and regulation of movement.

The incertohypothalamic dopaminergic pathway has been shown to play a crucial role in the regulation of sexual behavior (Melis and Argiolas, 1995). The tubero infundibular dopaminergic pathway is involved in the regulation of reproductive processes, as well as controls prolactin secretion from the anterior pituitary gland (Weiner and Ganong, 1978).

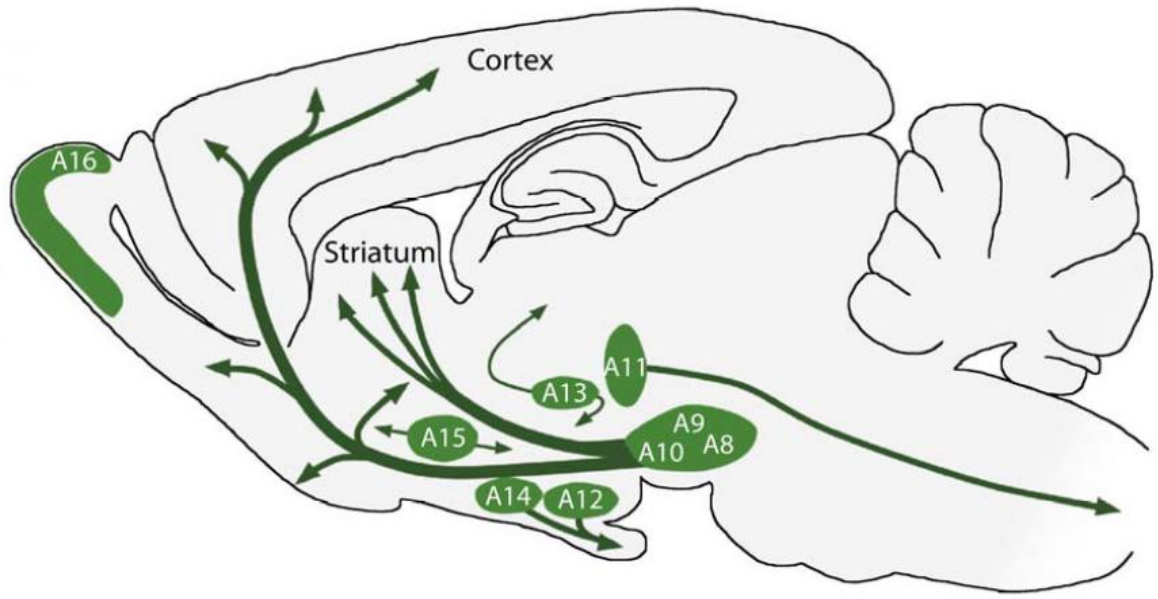


Figure 2: Distribution of dopamine neuron cell groups in rat brain. Adapted from Melis and Argiolas, 1995

An important component in dopamine signaling is the dopaminergic receptor. There are five different types of dopamine receptors D1-D5, all of which are members of the G-protein coupled

receptor super-family. But, they are subdivided into two families of dopamine receptors, the D1R (D1 and D5) and D2R (D2, D3 and D4) based on the G-proteins with which they couple, as well as their sequence homology (Siegel, et al., 2006).

D1R, comprising the D1 and D5 receptors, are coupled to Gs/q-α subunit, and result in stimulation of adenylyl cyclase and cAMP production, whereas D2R, comprising the D2, D3, and D4 receptors, couple to Gi/o proteins and result in the inhibition of adenylyl cyclase and suppression of cAMP production (Wang et al., 1995, Siegel, et al., 2006). D1R, by stimulating cAMP production, are excitatory, whereas activation of D2R is inhibitory.

Dopamine receptors are found in many brain areas and peripheral tissues. The five different dopamine receptors show unique patterns of regional distribution in the human brain (Abi-Dargham and Moore, 2003). D1 receptors are exclusively expressed on postsynaptic neurons, while D2 receptors are expressed on both presynaptic and postsynaptic neurons. In fact, D2 receptor is the main presynaptic autoreceptor of the dopaminergic system. D1/D2 receptor heteromers have been implicated in various pathologies with unique signaling properties (Rashid et al., 2007). D1 receptors are densely expressed in PFC and striatum, but are also expressed in the amygdala, olfactory bulb and cerebellum. D2 receptors are present in high densities in the striatum, but at very low densities in the PFC. D1 and D2 receptors are differently distributed in the rat PFC. Generally, more D1 than D2 receptors are expressed in pyramidal and gamma-aminobutyric acidergic (GABAergic) neurons with little overlap between receptor types (Santana et al., 2009). There is some evidence that D2 receptors are diffusely distributed across cells, whereas D1 receptors are more located in membranes (Voulalas et al., 2011). A separation between D1 and D2 receptors was also reported for the hippocampal region with highest

expression in the enthorinal cortex and layer specific segregation. The CA1 of the hippocampus is rich in D1 but not of D2 receptors (Köhler et al., 1991). D3 receptors are found postsynaptically, and have a higher density in limbic areas of the brain, such as the NAC, while D4 receptors are located in the PFC and hippocampus. D5 receptors are present in the hippocampus and entorhinal cortex. The diversity and number of dopamine receptors expressed at a given synapse help to define the response elicited when dopamine is released. Dopamine transporter also plays an important role in directly regulating dopamine signaling, it also influences several components of the dopaminergic synapse. Therefore changes in the activity and functioning of dopamine transporter can markedly disrupt dopamine neurotransmission.

1.4.2. Dopaminergic control of working memory

Working memory is regulated by various modulatory neurotransmitters, particularly dopamine. Several studies support the importance of dopamine in working memory. The mesocortical dopaminergic pathway from the VTA to PFC is the system most supposed to modulate working memory activity. Lesioning of the mesocortical pathway by 6- hydroxydopamine (6-OHDA) in the DLPFC results in impaired spatial working memory performance in primates (Brozoski et al, 1979). The impairment induced by dopamine depletion was subsequently reversed by administration of levodopa (the immediate precursor to dopamine in the synthetic pathway) or apomorphine (a combined D1 and D2 receptor agonist) (Brozoski et al, 1979; Stam et al, 1989). In subsequent studies impairment in working memory was also observed in rats after 6-OHDA lesions of the PFC (Bubser and Schmidt, 1990; Simon, 1981).

Several other lines of evidence showed that there is an increase in extracellular dopamine in the PFC during working memory tasks, which exerts its actions via local D1 receptors (Sawaguchi and

Goldman-Rakic, 1991, 1994; Murphy et al., 1996; Collins et al., 1998; Robbins, 2000; Seamans and Yang, 2004; Arnsten et al., 2010). Electrophysiological studies in Monkeys (Sawaguchi et al., 1988) also further supported the importance of dopamine receptor stimulation to PFC for spatial working memory function.

Studies in human subjects have also provided evidence that working memory is modulated by dopaminergic transmission (Mattay et al, 2000). A number of human studies using relatively non-selective agents such as amphetamine and methylphenidate (Seeman and Madras, 1998) have resulted in improvement of working memory, although the improvement seems to be greater for those individuals who have relatively worse baseline performance. These agents are not selective for dopamine system, as they influence neurotransmitter systems other than the dopamine system. Additional evidence comes from studies of individuals with neuropsychiatric disorders associated with dopamine malfunction. Patients diagnosed with schizophrenia (Okubo et al., 1997), attention deficit and hyperactivity disorder (Russell et al., 1995), and Parkinson's disease (Bradley et al., 1990) often manifest working memory disabilities.

Several studies in non-human primates also suggest that optimal dopamine function is critical for working memory performance. Cortical dopaminergic transmission has been found to act in an inverted U-shaped manner. Deficits in working memory can be induced by either inflated or deficient dopaminergic transmission (Zahrt et al., 1997; Cools and D'Esposito, 2011) mainly by postsynaptic effects in the PFC (Williams and Goldman-Rakic, 1995; Seamans and Yang, 2004; Vijayraghavan et al., 2007; Cools and D'Esposito, 2011). Imbalanced receptor activations can induce opposite effects on working memory compared to within-range levels (Luciana et al., 1992; Bushnell and Levin, 1993; Murphy et al., 1996; Cai and Arnsten, 1997; Wilkerson and Levin,

1999). For example administration of low dose dopamine agonists can improve working memory in monkeys, especially those with impaired performance associated with factors such as advanced age (Arnsten et al; 1998; Cai and Arnsten ;1997). Thus, working memory function in individuals (or animals) whose baseline level of dopamine function is already optimal may be impaired by the administration of dopamine agonists, and may even in some cases be improved by the administration of dopamine antagonists. In contrast, working memory function in individuals or animals with reduced dopamine function in PFC (e.g. people with schizophrenia, healthy aging) may show more evidence of improved function with the administration of dopamine agonists.

Studies conducted in rodents and non-human primates suggest that dopamine exerts its modulatory effects on working memory primarily via the D1 receptor. For example D1 receptors in the PFC critically influenced performance accuracy on various working memory tasks (Cai and Arnsten, 1997). Furthermore, various D1 receptor antagonists impaired working memory performances in monkeys tested with occulomotor delayed response task as compared to injections of saline and D2 receptor antagonists (Sawaguchi and Goldman-Rakic, 1991, 1994). In another studies conducted by Castner, et al. (2000), D1 agonists effectively reversed the working memory decline observed during and after long periods of haloperidol administration in monkeys. A study conducted on rodents (Seamans, et al., 1998) further support the greater influence of D1 receptors on the functioning of working memory in the PFC.

Studies of the D2R (D2, D3, D4) have resulted in inconsistent, and sometimes conflicting results. Studies in monkeys have shown that systemic administration of raclopride (a D2 antagonist) significantly decreased working memory task performance as compared to SCH23390 (a D1R antagonist) administered groups (von Huben, et al; 2006). Working memory impairment was also

observed in monkeys chronically administered with the potent D2 receptor antagonist, haloperidol (Castner, et al; 2000). Acute challenge with various D2R antagonists has been reported to disrupt working memory and delay specific PFC neuronal activity in some studies (Murphy et al; 1996; Arnsten and Goldman-Rakic, 1998). D2R antagonists were also able to reverse working memory induced by physiological stress (Arnsten and Goldman-Rakic, 1998). In Human study bromocriptine (Luciana et al; 1992) enhanced spatial but not object working memory. Thus, it is possible that D2R could play a specialized role in controlling the spatial components of working memory. Neuroimaging studies suggested that D2 receptor modulating agents could exert their effects on spatial working memory outside of the PFC, specifically in the hippocampus (Takahashi, et al; 2007, 2008).

1.5 Assessing Spatial working memory in Animals.

In animal cognition working memories are based on events from a specific trial, and reference memories are formed over repeated trials from the unchanging circumstances of a task (Honig, 1978). Animal models of working memory are designed to assess processes analogous to those identified in human subjects. Procedures such as delayed match-to-sample (DMTS) and delayed non-match-to-sample (DNMTS) tasks have been used with a variety of species to demonstrate patterns of forgetting (loss of stimulus control) across delays that are comparable to patterns observed in humans (Wright, 2007). Variants of DMTS/DNMTS procedures, along with tasks such as the radial arm maze and within-session Morris swim task, have been used specifically as models of working memory (Dudchenko, 2004). The standard tests for visuospatial working memory are delayed response and delayed alternation tasks (DRT and DAT), where as those for non-spatial visual working memory are DMTS and delayed object alternation tasks.

In non-human primates, working memory has been studied extensively since the 1930s with DRT, first developed by Jacobsen (1935). A prototypical DRT involves the presentation of a stimulus, followed by a short delay period and the subsequent presentation of a set of alternative choices. The animal can select the correct stimulus among the alternatives if it can remember both the rules of the task and enough information about the location of the correct stimulus. The entire process calls for the existence of internal representations and the animal's ability to use that representation to act appropriately in the absence of external cues. This ability requires the animal to filter out or inhibit irrelevant information and instead focus on the main task.

Delayed alternation is one of the most used tasks to test working memory in rodents. For example in a T-maze, rats are placed at the base and allowed to visit an arm to retrieve a food reward. Then, in a subsequent trial, animals are placed once again at the base of the maze and must enter the arm it had not entered before. Rats must remember their previous location in order to select an alternative response and to alternate. It has been found that rodents and in particular rats have a natural tendency to alternate their choices on repeated trials (Tolman, 1925). When the rat alternates, it remembers the previously visited arm based on the extra maze spatial cues and uses this memory to correctly solve the task. It is assumed that rats solve the T-maze by remembering the location of the most recently visited arm based on its spatial relationship with extra maze landmarks. This is an allocentric (world-based) spatial memory. The rat, however, may alternate based on a directional sense, first going west, for example, and then going east. Alternatively, the rat might use a response strategy remembering which turn it has made (e.g. left), and make the opposite turn on the subsequent trial. Therefore, the rat could use a variety of strategies to remember which arm they have entered most recently.

Radial arm maze tasks are also used to assess this form of one trial learning (Olton, 1979). In this task, rodents are placed in the center of an open field apparatus that has a number of arms extending from a central platform. Each of these arms is baited with a food reward and the rat is able to explore the apparatus and consume the rewards. Any time the subject re-enters an arm which it has already explored an error is scored. In optimal performance of the task the rat enters each arm only once. During a trial, the subject must remember which arms it has visited; errors are interpreted as a failure of memory.

A delayed match-to-place task can also be used to assess working memory in rats (Runyan et al., 2005). For example in water maze task, rats are allowed to find the location of a hidden platform in a water maze; then , after a brief delay, are required to find it once again. Following an inter-trial interval, the location of the hidden escape platform is moved and the task is repeated. The task can be repeated with in a session. The decrease in time between the first (naive) and second (experienced) trial is used as an indicator of the animal's capacity for working memory. Similar to other working memory tasks, either too much or too little dopamine also impairs performance in this task (Williams and Goldman-Rakic 1995; Zahrt et al. 1997; Runyan et al. 2005).

1.6 Rational for the study

Dopamine has been identified to regulate working memory within a narrow range of concentration. Dopamine and working memory appear to exhibit an inverted U-shaped relation, suggesting deficits in working memory by either elevated or deficient cortical dopaminergic transmission could be observed (Zahrt et al., 1997; Cools and D'Esposito, 2011).

Patients with severe perturbations of this balance like as in neuropsychiatric disorders, such as Parkinson's or Alzheimer's disease often manifest working memory disabilities (Bradley et al., 1989; Beato et al., 2008). Levodopa therapy in humans and animal models had a positive effect by increasing extracellular dopamine levels, not only on related motor dysfunction but also on spatial working and reference memory tasks (Costa et al., 2003; Beato et al., 2008; Ambree et al., 2009; Ruocco et al., 2014; Trossbach et al., 2014), although no effects of dopaminergic medications on spatial working memory in Parkinson's disease could be determined in some studies (Lange et al., 1995; Fournet et al., 2000). A similar effect has been observed after application of other dopamine targeting drugs such as modafinil. Modafinil inhibits the dopamine transporter that facilitates the reuptake of extracellular dopamine in the synapses. Thus, both drugs increase the level of extracellular dopamine though by different mechanisms, diffusion of exogenous dopamine through levodopa and inhibition of the reuptake of endogenous dopamine through modafinil. However, the effects of repeated application of these drugs on the repetitive updating of spatial working memory during training are still unclear. Therefore, the effects of two dopaminergic transmission targeting drugs (modafinil and levodopa) were investigated on spatial working memory in rats trained over six days in a delayed alternation T-maze task (a commonly used paradigm to assess spatial working memory in rodents) (Dudchenko, 2004). Temporal effects, in terms of subsequent training sessions, of dopamine receptors on working memory are also rarely reported. Thus, training was also conducted in the presence of agonists and antagonists of the D1R and D2R to decipher the role of these receptors. Optimal stimulation of dopamine receptors appears to be necessary for optimal working memory performance in rodents and primates in acute treatment. However, the range of receptor activation or dopaminergic transmission required to

modulate spatial working memory may vary at different status of training up on repeated application of drugs. The present study also investigated the pattern of variation of spatial working memory modulation at different stages of training. Several tasks can be used to assess spatial working memory modulation but there could be differences in complexity of the tasks for the animals in spatial working memory performance. Hence task dependent effects of spatial working memory modulation were assessed using T-maze and water maze tasks. cAMP is a secondary messenger system up on dopamine receptor activation for D1-like receptors. D1R, comprising the D1 and D5 receptors, are coupled to Gs/q-α subunit, and result in stimulation of adenylyl cyclase and cAMP production, whereas D2R, comprising the D2, D3, and D4 receptors, couple to Gi/o proteins and result in the inhibition of adenylyl cyclase and suppression of cAMP production (Wang et al., 1995, Siegel, et al., 2006). The change in cAMP level up on repeated application across training using different concentration range of dopamine agonists and antagonists were also investigated.

2. Objectives

2.1 General objective

- To evaluate dopaminergic modulation of spatial working memory in rat.

2.2 Specific objectives

- To evaluate the effect of repeated application of dopaminergic transmission targeting drugs (modafinil and levodopa) on spatial working memory in rats.

- To evaluate an interaction of dopaminergic treatment effect with training induced improvement on spatial working memory performance.

- To determine specific dopamine receptors involved in temporal modulation of working memory.
- To test the task dependent effect of spatial working memory using T-maze and water maze tasks.

- To determine the change in cAMP level up on using different concentration range of dopamine agonists and antagonists.

3. Materials and Method

3.1. Chemicals and drugs

Levodopa and carbidopa (Sigma-Aldrich), receptor agonists (D1R: (±)-6-CHLORO-PB hydro bromide (SKF-81297, Ki D1: 2.2nM); D2R: Sumanirole maleate (Sigma-Aldrich, S143 and SML1087, respectively; Ki: D2:9.0 ± 1, D3: 1940±142; D4:>2190, D1: >7140, nM) and antagonists;D1R: (R+)-SCH-23390 hydrochloride (Sigma-Aldrich, D054; Ki: D1: 0.11-0.35; D5: 0.11-0.54; nM); D2R :Remoxipride hydrochloride (Tocris, 0916; Ki: D2: 54-300; D3: 969-1600; D4:2800-3690 nM) were used for the experiments.

Ki values were taken from Anderson and Jansen (1990); Vallone et al. (2000) for SCH and Remoxipride (min/max values), McCalletal. (2005) for Sumanirole

3.2 Animals

The study was conducted using male Sprague–Dawley rats (12–13 weeks old). They were bred and maintained in cages made of Makrolon filled with autoclaved woodchips in the Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna. One week prior to the behavioral tests animals were moved to a separate experimental room where they lived throughout the experiment. Rats were housed individually in cage at (temperature: 22 ± 2◦C; humidity:55 ± 5%; 12 h artificial light/12 h dark cycle: light on at 7:00 am).The study was carried out according to the guidelines of the Ethics committee, Medical University of Vienna, and were approved by the Federal Ministry of Education, Science and Culture, Austria.

3.3. Handling and habituation

A total of 177rats were included in the experiment. Sixty nine rats were used for experiment with I.P administration of levodopa and modafinil with 6 days training in T-maze and 81rats for the experiment with ICV administration of D1R and D2R Agonist and Antagonist for 3 days training in T-maze and 27 rats for water maze task. All the rats were handled for 15 min each day for 3 consecutive days before habituation. The body weight of the animals was recorded from first day of handling throughout the experiment. The animals were mildly deprived of food during this period to decrease body weight to 85% of free feeding weight while the tap water was given adlibitum. The body weight of the animals was maintained to 85%of free feeding weight by providing them with limited amount of pellet daily. Cage controls were also food-deprived for the same time period as trained animals and kept in their home cages in the experimental room.

Animals were habituated to a T-maze until they voluntarily ate a piece of pellet placed at the end of each arm. Food reward pellets were provided in the home cage each day for a few days prior to training in order to familiarize to the reward. Habituation was carried out on the fourth and fifth day of food deprivation. During this habituation period, all animals were allowed a 15-min free exploration of the apparatus, daily for two days to familiarize them with the experimental conditions. On the first day of habituation pellets were kept throughout the maze and on the second day only in the food cups located at the end of both arms. After free exploration of the apparatus the animals were carefully picked up and kept back to home cage. For water maze task before the 3 day training phase rats were handled 2 days for 15 min each, and thereafter habituated to the pool at the following day by a 90s swim without platform.

3.4. Surgery

The rats were anesthetized with Nembutal (40 mg/kg, i.p.). An ICV cannula (4.5 mm in length) was stereotactically implanted into the lateral ventricle of the right hemisphere (coordinates: AP -0.8; L -1.5 from bregma). Together with an anchor screw the cannula was fixed with dental cement (Paladur, Heraeus Kulzer, Hanau, Germany). The animals were allowed to recover from surgery for at least 4 days. The correct placement of the cannulas were tested by an angiotensin II (70 ng/µl; 5µl volume) drinking test (drinking within 3 min). From 97 rats 16 rats failed to drink, 81 rats were included in the experiment of D1Rs and D2Rs agonist and antagonist treatment in T-maze task. Those failed rats were discarded

3.5. Drug administrations and training

Two mg or 20 mg/kg of a levodopa and carbidopa mixture in a ratio of (4:1) dissolved in saline, or 1 mg and10 mg/kg of modafinil dissolved in 100% DMSO were applied with five minutes delays between trials during which rats were placed in a cage. All the drugs and vehicle control (saline and DMSO) were administered I.P 30 min prior to the start of behavioral testing for days 6-11.

The receptors agonist and antagonist were dissolved in saline and applied at a volume of 5 µl at a rate of 1 µl/min using a Hamilton syringe (CR700-20) by ICV route at a dosage of 1µg and 5µg 30 minutes prior to each training sessions at day 6,7 and 8. Saline treated rats served as controls. Doses were chosen because of previous own and literature experiments that show these doses affect learning and memory. Saline and not artificial cerebrospinal fluid was used as control substance because it was the dissolvent for the drugs. In water maze task, similar as for the T-maze

animals were infused ICV with the D1Rs agonist at a dose of 1µg, 5µg and saline controls 30 min prior to training.

3.6 T- maze studies

The T- maze (black acrylic) consisted of two goal arms (50 cm long, 10 cm width, with wall height of 25 cm). The starting arm (70 cm) was equipped with a starting box (20 cm in length) separated from the maze by a guillotine door. At the edge of each goal arm, there was a small cup (to prevent rats from seeing whether the dish was baited) containing highly palatable food pellet (dustless precision pellets, 45 mg, Bio-Serv, Frenchtown, NJ; USA). A large amount of food pellet was also placed outside both goal arms to mask olfactory cues. The maze was located in the same position in a room with several easily identifiable visual cues (equipment, walls, doors), and cleaned with 1% incidin® between each animal in order to remove any olfactory cues. Indirect illumination by floor positioned lamps directed to the ceiling provided equal light intensities in each arm. Trials were monitored by a camera fixed to ceiling and videos stored at a computer.

A delayed none matching to place task was performed. In T- maze task each training session consisted of 10 trials (a forced trial followed by 9choice trials). To begin a trial, the rat was placed in the starting box for 15 s, before the guillotine door separating the starting box from the main alley was raised immediately and opened. In the forced trial, a randomly selected goal arm was blocked by a guillotine door, and a reward was placed in the opposite arm, hence the rats were forced to visit a baited arm.

In choice trials, both arms were accessible, but reward was available only in the arm not entered in the previous trial. In the choice trials 1 through 9, rats had to avoid the arm once visited in a

previous trial and select the opposite arm to get reward. The next trial began after an interval of 5 min delay. Once the animal has chosen an arm, it was allowed about 10 s to consume the pellet. Arm entries were recorded when the whole animal, including the tail tip, was in the arm. If rats selected the un-baited arm, a self-correction procedure was introduced by keeping the baited one still baited until it was visited, giving the rats a chance to shift their choice. Entry into the arm visited in the previous trial was registered as working memory error (WME). In addition, the working memory index (WMI) was calculated (correct choices/total trials).

3.7 Water maze studies

The maze consisted of a black circular plastic pool (150 cm in diameter, 60 cm wall height) equipped with a black quadratic escape platform (10 cm x 10 cm) at a height of 38 cm. The pool was filled with water (25 ± 2° C) up to 39.5 cm. Platform positions were located halfway between the wall and the middle of the pool in four quadrants. Five different positions were used each day for the sample trials (90s to discover the platform) followed 5 min later by a test trial (90 s) for recall of the platform position. In case the animals did not find the platform during the sample trial, they were guided by hand and allowed to remain for 15 s onto it. Starting positions for sample and test trials varied pseudo randomly.

In water maze task the time to reach the platform (escape latency), the distance travelled and the mean velocity was recorded by a tracking system (TIBE, V 1.0, Imagination, Vienna, Austria) and stored on a computer. For the analysis the mean of escape latencies and velocities of the 5 test trials for each rat/day was calculated. These values were then statistically analyzed between groups. All behavioral training/testing was performed during the light phase of the light–dark cycle.

3.8 Determination of cAMP levels

Rats treated with D1R and D2R agonist and antagonist trained in T-maze were killed with CO2 inhalation, decapitated, brains rapidly removed and the frontal cortex dissected on a Para Cooler (RWW Medizintechnik, Hallerndorf, Germany) at 4 °C. The CO2 exposure duration till point of death and then the duration from exposure to CO2 and brain removal were similar for each rat. The rat tissue samples were homogenized in ice-cold hydrochloric acid (0.1M) and centrifuged at 1000Xg for 30min at 4°C. Subsequently, 100 µL of the supernatant was assayed with a commercial cAMP ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) following the manufacturer's instructions. The cAMP concentration was normalized to the total protein content.

3.9 Statistics

Data are presented as arithmetic means and standard errors for parametric data. In order to address the high variability in day to day performance in levodopa and modafinil treated rats, a one-way-ANOVA with Tukey post hoc tests was conducted for each day. As an indication of learning linear regression analyses were performed for mean working memory indices and days of training.

A repeated measure ANOVA with Tukey post hoc tests for the differences over the entire training and Bonferroni post hoc tests for differences at specific days was conducted for dopamine agonist and antagonist treated rats. Within group performance over training days were tested by T-test for connected samples. Differences in cAMP levels were tested with the Kruskal-Wallis-test and subsequent Mann-Whitney-U-tests because this data is nonparametric. SPSS Statistics software version 20 was used for statistical analysis. Border of significance was set at $p \leq 0.05$.

4. Results

4.1 Effect of levodopa and modafinil on working memory in T-maze

4.1.1 Modafinil

Modafinil at both high and low doses did not show a significant working memory performance as compared to DMSO controls at any day (Figure 3). However group differences in WME ($F_{2, 31}$= 3.37, $p = 0.047$) and close to the border of significance in WMI ($F_{2, 31}$= 3.16, $p = 0.056$) was observed at day 3. The low dose treated groups (1 mg/kg) ($n = 10$) showed better performance as compared to high dose (10 mg/kg) ($n = 12$) treated group ($p < 0.05$) as revealed by the post hoc analysis. Only the DMSO treated control group ($n = 12$) showed a significantly positive linear regression of WMI over days indicating a learning improvement during training. A deviation from the linear model could not be detected (Runs test: $p = 0.90$). Both high and low dose treated groups did not show a constant learning performance over the training procedure.

4.1.2 Levodopa

Like modafinil, significant group differences in working memory performance was also observed in levodopa treated animals in both WME ($F_{2, 32}$= 5.67, $p = 0.007$) and WMI ($F_{2, 32}$= 5.67, $p = 0.007$) only at day 3 (Figure 3). Unlike modafinil, the post hoc analysis revealed significantly enhanced working memory performance in animals treated with a higher dose (20 mg/kg, $n = 11$) over those treated with a lower dose (2 mg/kg, $n = 12$). Moreover, unlike modafinil, the high dose group showed better working memory performance than normal saline treated control animals ($n = 12$) as revealed by the post hoc tests ($p < 0.05$ both WMI and WME). However, considering the entire training period, only the normal saline treated control group showed a significantly positive

linear regression of WMI (Runs test: $p = 0.70$). A statistically significant positive linear regression of WMI and days of training was not observed for both high and low doses of levodopa treated groups.

Taken together no significant working memory improvement was induced by modafinil but by levodopa. Dose-related effects on working memory were opposite between the two drugs and only effective during the early training. Performance decreased and remained at control levels during late training. Constant day to day improvement over training could be observed only in both vehicles (DMSO and normal saline) treated groups.

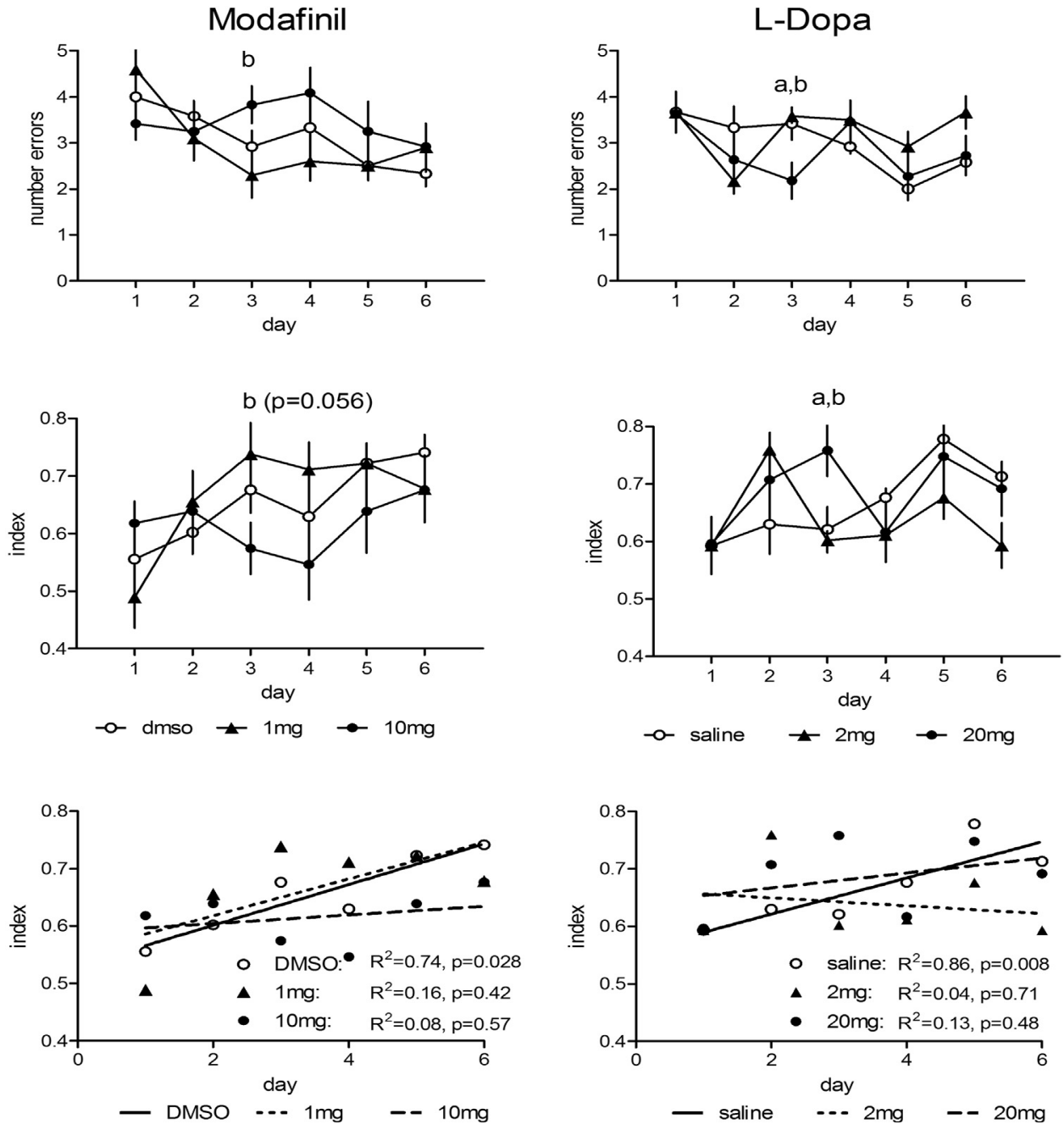


Figure 3: **Performance in a T-maze task following administration of dopaminergic agents:**

Numbers of working memory errors (upper panel) and working memory indices (middle panel) for Modafinil (left panel) and levodopa (right panel) treated rats. The lower panel was for the vehicle (DMSO) (left panel) and saline (left panel) treated rats. Significant differences between groups appear at day 3 for

both drugs. a: statistically significant differences of drug treated as compared to vehicle treated rats. b: statistically significant differences between groups treated with high and low doses of the drugs. Statistically significant linear regressions (lower panel) between working memory indices and days of training could be determined only in vehicle treated groups in both experiments, indicating a constant improvement of working memory only in the control groups.

4.2 Effect of dopamine agonists on working memory in T-maze

Regarding the receptor agonist and antagonist treated groups a significant difference was revealed in day x treatment interaction in both WMI ($F.14, 146/ = 2.27$, $p = 0.008$) and WME ($F.14, 146/ = 2.17$, $p = 0.011$). Similarly, a significant treatment effect in the WMI ($F.7, 73/ = 5.79$, $p < 0.001$) and WME ($F.7, 73/ = 5.75$, $p < 0.001$) was observed (Figure 4).

4.2.1 D1R Agonist

Significantly higher WMI ($p = 0.007$), and less WME ($p = 0.008$) was noted by D1R agonist treated rats at a dose of 1 μg compared to normal saline treated rats in over all training. Day specific analysis revealed a higher index in rats treated with1 μg as compared to controls at day 2 ($p < 0.05$) and day 3 ($p < 0.01$), whereas less WME, specifically at day 1 ($p < 0.05$) and day 3 ($p < 0.01$) was observed (Figure 4A).

Comparing rats treated with D1R agonist at dosage of 5 μg to control animals yielded no detectable differences in both indices ($p = 0.56$) and WME ($p = 0.56$) throughout the training, whereas day specific analysis showed significantly reduced errors ($p < 0.01$) and higher indices ($p < 0.01$) only at day 1(Figure 4A).

4.2.2 D2R Agonist

Rats treated with D2R agonist at both 1 and 5 μg dosages did not show difference in performance over training both in WMI ($p = 0.99$ each dose) and WMI as compared to controls. Day Specific analysis also revealed no differences at any day (Figure 4B).

4.3 Effect of dopamine antagonists on working memory in the T-maze

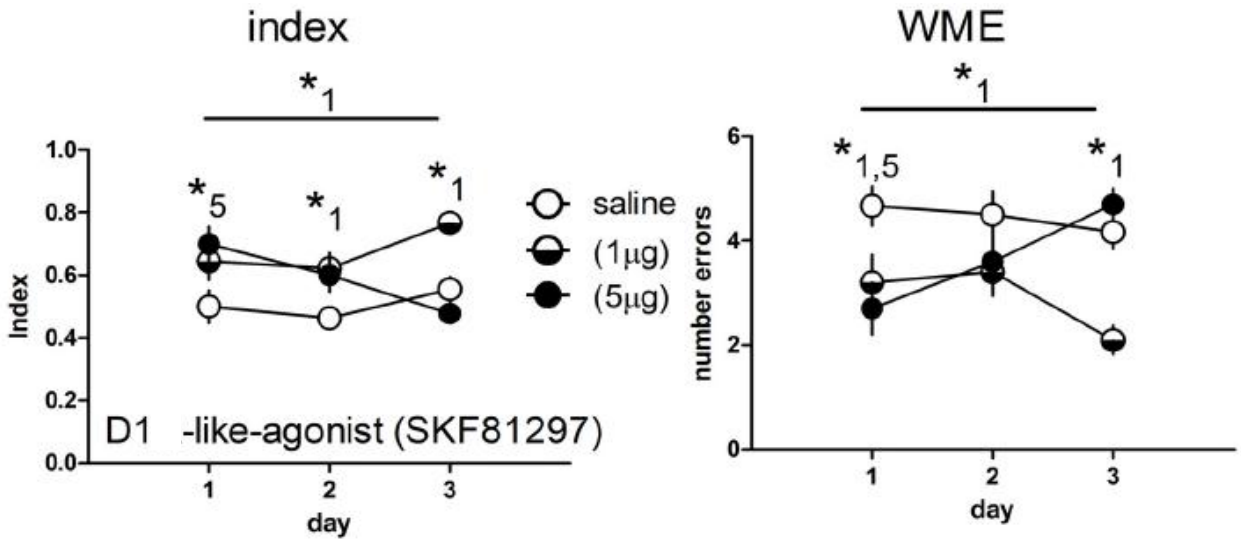
4.3.1 D1R Antagonist

D1R antagonist treated rats at a dose of 1 μg did not show differences in WMI and WME throughout the training as compared to saline treated controls ($p = 0.35$, each). Day specific analysis also yielded no differences in index at any day, whereas significantly reduced error was observed specifically at day 3 ($p < 0.05$) (Figure 4C).

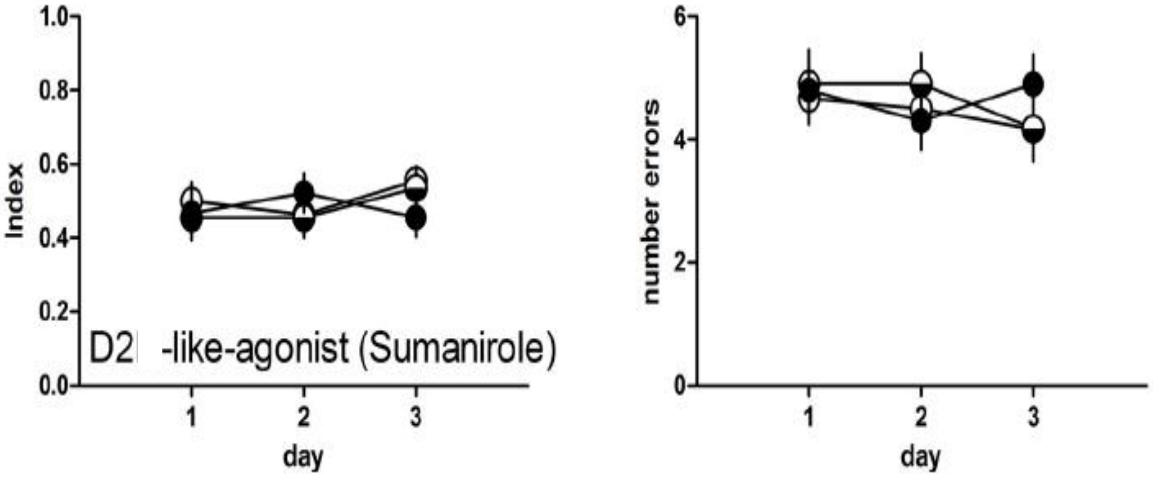
Rats treated with D1R antagonist (5 μg), showed significantly enhanced indices and reduced errors over training compared to saline treated ones ($p = 0.013$, each). Day specific analysis showed higher indices and lower error specifically at day 2 ($p < 0.001$ each) (Figure 4C).

4.3.2 D2R Antagonist

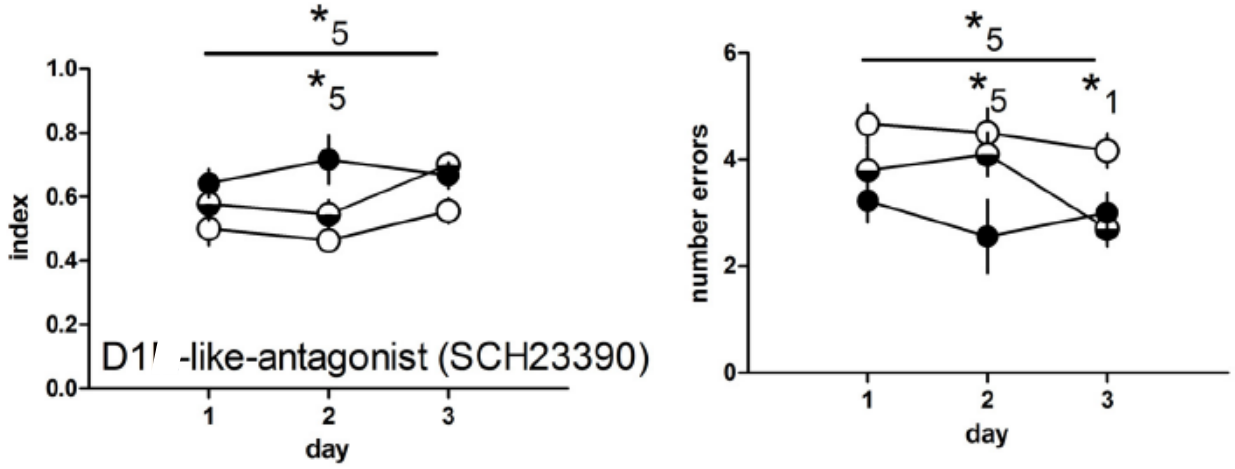
For D2R antagonist, rats were tested using only one dosage (1 μg), because no difference was revealed by D2R agonist in over training and day specific effect. D2R antagonist treated rats exhibited no overall difference neither in the WMI nor in the WME ($p = 0.42$, each) compared to controls. However the post hoc tests for day specific analysis showed significantly enhanced WMI ($p < 0.01$) and reduced WME($p < 0.05$) at day 2(Figure 4D).


index
WME
*1
*1
*5
*1
*1
*1,5
*1
saline
(1μg)
(5μg)
Index
number errors
D1 -like-agonist (SKF81297)
day
day

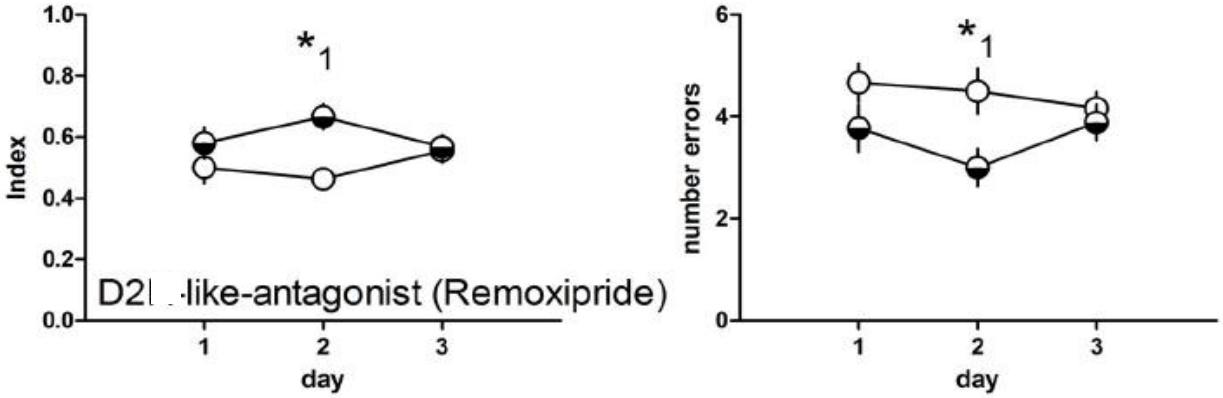
A.


Index
number errors
D2 -like-agonist (Sumanirole)
day
day

B.



C.



D.

Figure 4: Working memory indices (left panel) and numbers of working memory errors (WME, right panel) of rats treated with **(A)** a D1-like receptors agonist (n = 10 for each dose) and **(B)** D2-like receptors agonist (1 µg: n = 11; 5 µg: n = 10) and **(C)** D1-like receptors antagonist (1 µg: n = 10; 5 µg: n = 9) **(D)** D2 -like receptors antagonist: n = 9) or saline (n = 12). Significant differences between groups over the entire training are indicated by an asterisk above horizontal bars. Significant differences between groups

for specific days are indicated by asterisks above daily data points. Numbers give the drug dose (1: 1 µg; 5: 5 µg). Given are the mean value and SEM.

4.4 Working memory assessment using water maze

In T-maze experiments, the most effective drug found was D1R agonist. Hence, water maze experiments were performed only using D1R agonist at both doses and normal saline treated rats to compare the task dependent effect.

The overall analysis revealed no significant day x treatment interaction ($F_{4, 48}=1.43$, $p=0.239$) and no significant treatment effect ($F_{2,27}=1.46$, $p=0.253$) through training for the escape latencies as well as for swim velocity ($F_{4,48}=0.42$, $p=0.796$) and ($F_{2,24}=0.73$, $p=0.493$), respectively (Figure5).

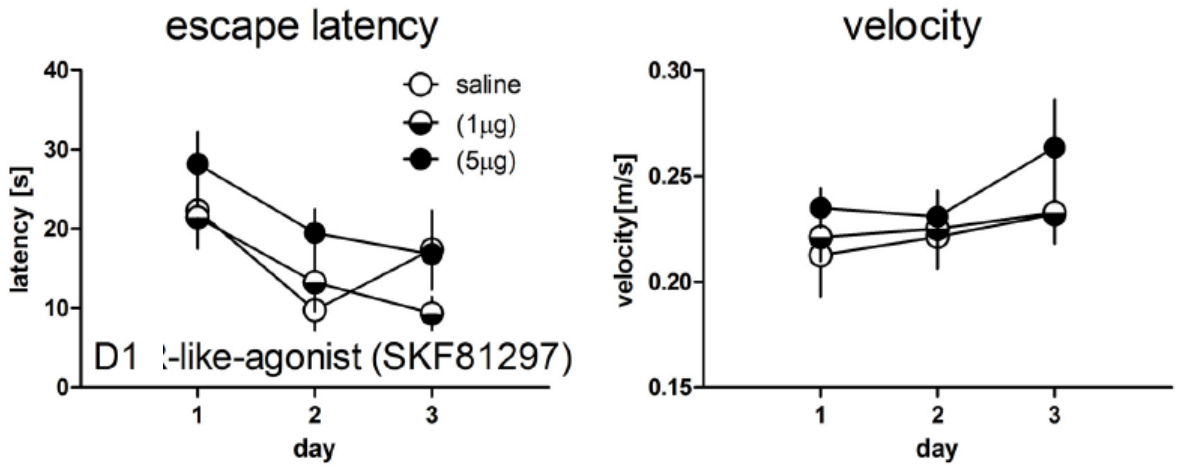


Figure 5: Escape latencies (left panel) and mean velocity (right panel) during test trials in the water maze working memory task in rats treated with saline (n = 9) or the D1 like receptors agonist at a dose of 1 µg (n = 10) or 5 µg (n = 8). No significant differences between groups could be detected. Given are the mean values and SEM.

4.5 Comparison of performance in the two paradigms

4.5.1 Within-group performance over training days

In D1R agonist treated rats, within group performance between days was different task dependently (Table 1). No significant difference in performance between days was observed in the T-maze control as well as the low dose treated rats. However, high dose treated rats performed significantly worse at day 3 compared to day 1 ($T=3.46$, $p=0.007$) but not between the other days.

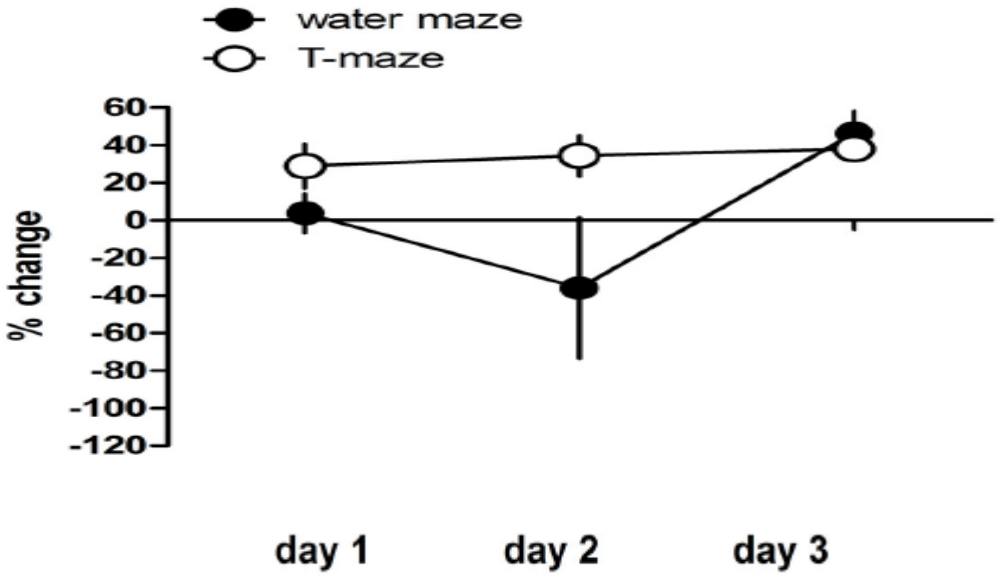
Unlike T- maze trained rats, difference in Performance in the water maze control rats (day 1- day 2: $T=3.06$, $p=0.016$; day 2 – day 3: $T=-2.37$, $p=0.045$, $df=8$) and low dose treated rats (day 1- day 2: $T=3.39$, $p=0.008$; day 1 – day 3: $T=-4.52$, $p=0.001$) was observed. In contrast to T- maze, high dose treated rats did not show significant differences in daily performance in water maze.

Table 1: Within-group performance of rats treated with saline (n = 9, water maze; n=12 ,T-maze) or the D1 like receptors agonist for water maze trained rats at dose of (1 μg ,n = 10; 5 μg ,n = 8) and T maze (n = 10 for each dose). Statistically significant differences in performance (WMI for T-maze and escape latency for water maze) between days are indicated by asterisks.

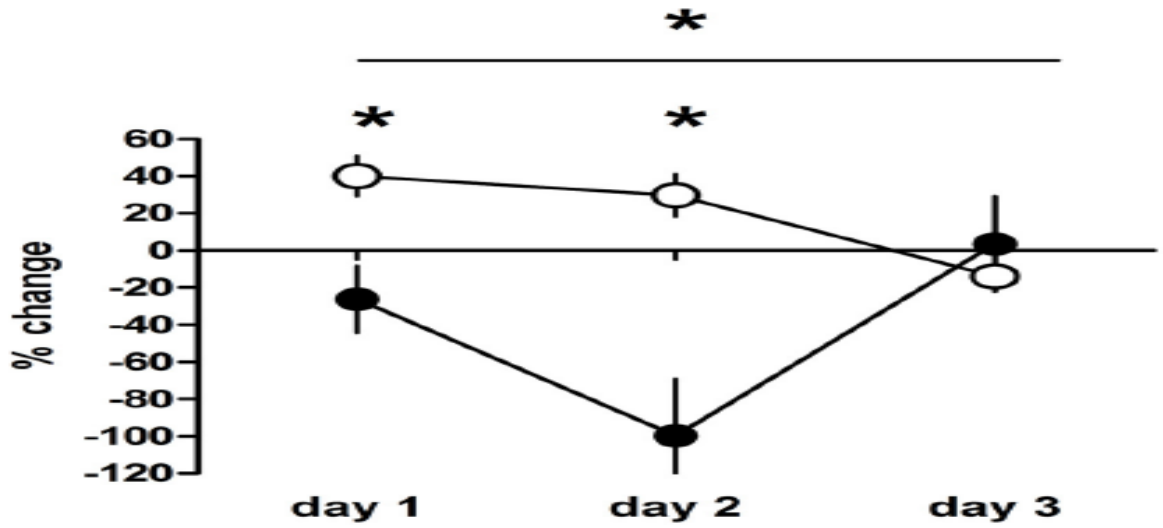
Task	Treatment group	Training Days	T	P	df
T -MAZE	CONTROL (Normal saline)	day1-day2 day1-day3 day2-day3	0.63 -1.15 -1.89	0.54 0.27 0.085	11
	LOW DOSE (1 μg)	day1-day2 day1-day3 day2-day3	0.29 -1.67 -2.25	0.78 0.13 0.051	9
	HIGH DOSE (5 μg)	day1-day2 day1-day3 day2-day3	1.09 3.46 1.77	0.3 **0.007 *** 0.11	9
Water MAZE	CONTROL ((Normal saline)	day1-day2 day1-day3 day2-day3	3.06 -1.36 -2.37	**0.016*** 0.21 **0.045***	8
	LOW DOSE (1 μg)	day1-day2 day1-day3 day2-day3	3.39 -4.52 1.17	**0.008*** **0.001*** 0.27	9
	HIGH DOSE (5 μg)	day1-day2 day2-day3 day1-day3	1.65 2.1 0.57	0.14 0.073 0.58	7

4.5.2 Comparison of D1R agonist effects between the two tasks

Because of the different units of behavioral recordings used in T-maze and water maze tasks it was not possible to directly compare the drug effects between the two tasks. Therefore, comparison was made using the deviation of the performance of individual drug treated rats expressed as percentage from the mean performance of the respective control group between rats trained in the T-maze or water maze (Figure 6). Changes in WMI (T-maze) and escape latencies (water maze) for both low and high doses of D1Rs agonist effects were used for comparison. Negative values indicate impairment and Positive values indicate improvement of working memory performance. The overall analysis revealed a significant day effect ($F_{2,68}=5.59$, $p=0.006$), a day x task interaction ($F_{6,68}=4.79$, $p<0.001$) and a significant task effect ($F_{3,34}=6.60$, $p=0.001$). The low dose treated rats showed no significant difference between T-maze and water maze tasks as revealed by Post-hoc tests ($p=0.307$), whereas high dose treated rats performed less in the water maze compared to the T-maze ($p=0.009$), specifically at day 1 ($p<0.05$) and day 2 ($p<0.001$) but not at day 3.



A. Low dose(1 μg) treated groups



B. High dose(5 μg) treated groups

Figure 6: Changes in working memory indices (T-maze) and escape latencies (water maze) expressed as variation in percentage from the mean value (100%) of the respective control group of individual D1R agonist treated rats at a dose of (A) 1 μg or (B) 5 μg. Positive values indicate improvement and negative deterioration of working memory. Significant differences between groups over the entire training are indicated by an asterisk above horizontal bars. Significant differences between groups for specific days are indicated by asterisks above daily data points. Given are the mean values and SEM.

3.6 cAMP determination

The data obtained from this measurement is presented in Figure 7. Subsequent analysis revealed that there was an overall significant difference between groups (Kruskal-Wallis-test: X^2 =36.3, df=8, p<0.001). Further analysis using Mann-Whitney-U-tests showed a significant difference between cage and vehicle controls (Wilcoxon-W= 78, U=23.0, p=0.015), vehicle controls and D1R antagonist (1µg) treated animals (Wilcoxon-W= 82, U=4, p=0.003) and vehicle controls and D2R agonist (5µg) treated rats (Wilcoxon-W= 105, U=27.0, p=0.030).

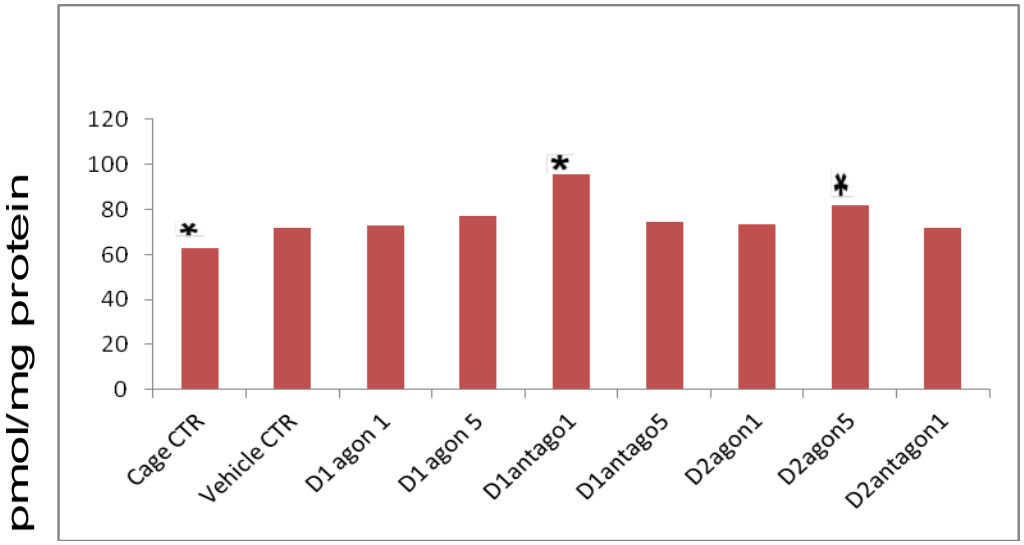


Figure 7: Levels of cAMP measurement in rats of all groups. Cage CTR, cage control; Vehicle CTR, vehicle control; D1 agon1, D1-like receptors agonist (1 µg) ; D1 agon 5, D1-like receptors agonist (5 µg) ; D1 antago1, D1-like receptors antagonist (1 µg) ; D1 antago5, D1-like receptors antagonist (5 µg) ; D2 agon1, D2-like receptors agonist (1 µg) ; D2 agon 5, D2- like receptors agonist (5 µg); D2 antgon1, D2-like receptors antagonist (1 µg). Statistically significant differences as compared to vehicle treated rats are indicated by asterisks above groups.

5. Discussion

Considering the entire training period in T-maze, constant day to day improvement across training was observed only in the vehicle treated (normal saline and DMSO) control groups. This is attributed only to the training related changes in working memory circuits and mechanisms underlying spatial working memory (Klingberg, 2010). However, both high and low doses of modafinil and levodopa treated groups did not show a constant improvement in learning performance over the training procedure. This variation in effect across days can be explained by interaction of training related changes and with effects related to receptor activation by drugs at different days and possible interaction between the signaling mechanisms of different dopaminergic receptors (D1R and D2R). Both modafinil and levodopa enhance extracellular dopamine level although by different mechanism. This common time-dependent underlying mechanism induced by both drugs could explain the similar effects (group differences in performances) produced by drugs at a specific time point during training (day 3). Since the common effect is the increase of extracellular dopamine, it is plausable to assume that this may depend on similar alterations of the dopaminergic system by chronically increased extracellular dopamine levels.

The high dose levodopa treated group but not modafinil group showed better working memory performance than normal control animals at day 3. In addition, the two extracellular dopamine enhancing agents showed group differences at the same time point, which appears to be dose related. The low dose treated groups showed better performance as compared to high dose in modafinil treated group and the opposite for levodopa treated group. This could partly be explained by the physiological differences. While modafinil blocks the dopamine transporter and

thus the reuptake of dopamine in the synapse, levodopa increases the dopamine level just by diffusing into the brain. Modafinil in addition also targets the noradrenalin and serotonin transporter to a certain extent. The multiple effect of modafinil, attributed to its action on other systems (noradrenergic and serotonergic systems), could be enhanced with increasing dose. This could then possibly lead to interference with each other that culminates with impairment of the neuromodulatory machinery of working memory circuits.

To determine the contribution of each dopamine receptors, the T- maze test was conducted using D1R and D2R agonists and antagonists. Dopamine receptor activation dependent modulations of spatial working memory differ in a receptor specific manner. In D1R agonist treated groups, the effect produced vary in dose dependent and session specific manner. In day 1 or first day training, improvement in spatial working performance was revealed in high dose treated group, whereas no change was observed in low dose treated groups. This finding is in line with the previous studies, that optimum range of receptor activation is required to enhance spatial working performance (Murphy et al., 1996; Cai and Arnsten, 1997; Wilkerson and Levin, 1999). The relationship between D1 receptor activation and performance is nonlinear and inverted-U–shaped, with both excessive as well as insufficient levels impairing performance, whereas an optimum dose facilitates it (Williams and Goldman-Rakic, 1995). Therefore the high dose used in the present study was in the range required to produce optimum receptor activation, whereas the low dose used might not be sufficient to improve the spatial working memory. In contrast, in subsequent training days (day 2 and 3) no change in performance of high dose treated groups, but performance enhancement in low dose treated groups. This is because at such training status working memory underlying mechanisms and circuitries would get strengthened to certain level. Hence, making only minor additional activations of receptors by the agonist would be required to enhance

performance. Therefore, in such trainings, low dose appears to be sufficient to improve performance and high dose results in overstimulation of receptors, which would result in deterioration of spatial working memory performance.

The decline in performance of high dose treated groups in subsequent days could also be due to repeated administration of the drug and it is in line with previous studies (Jayms etal., 2006) that showed a decrease in performance as a result of dopamine enhancing agents like amphetamine mediated D1-inhibition of voltage-gated sodium currents in the PFC..

No improvement in working memory performance was produced by D2R agonist treated rats in both doses at specific days or across training. This finding is consistent with a previous study conducted with systemic administration of D2 agonists (Packard and White, 1989). Several lines of evidence indicate that effect of D2 receptor modulation on spatial working memory is more robust when experimental manipulations target the hippocampus rather than the PFC (Wilkerson and Levin, 1999; Takahashi, et al; 2007, 2008). ICV route was used in the present study, which made the drug to diffuse in various brain regions. Diffusion of these agents could lead to activation of dopamine autoreceptors, which are primarily D2 receptors in PFC and some D3 receptors, that results in dampening of the synthesis and release of dopamine as well as firing of dopamine neurons in regions other than hippocampus. Indeed, D2 agonists tend to have higher potency on autoreceptors-mediated effects compared with the postsynaptic D2 effects (Feenstra et al., 1983).

In the present study, D1R antagonists improved working memory performance specifically on day 2 and across training in high dose treated groups. This finding is in consistent with previous studies conducted in Y maze (Rusu et al., 2014) and radial arm maze (Rusu et al., 2013). Pretreatment with D1R antagonists (Zahrt et al., 1997) were also able to reverse spatial working

memory impairments due to excessive D1 receptor agonists. However, in contrast to the present study working memory was impaired at all drug concentrations and all test delays used, however session (day) specific effects were not determined (Bushnell and Levin, 1993; Wilkerson and Levin, 1999).

D2R antagonists also improved spatial working performance on specific day 2, but no improvement across training. Thus, the finding supports the greater influence of D1R blockage on the functioning of working memory. Day 2 seems sensitive time point for working memory modulation by blockage of both D1R and D2R.

In general, the main modulator of spatial working memory was determined to be D1R mechanisms and D2R mechanisms appeared to be less involved. The findings in the present study further support the view that spatial working memory is optimized within a limited range of dopaminergic transmission (Aultman and Moghaddam, 2001; Williams and Castner, 2006; Avery and Krichmar, 2015).

Those agents agonize or antagonize dopamine receptors showed group differences at different time points as compared to extracellular dopamine enhancing agents (modafinil and levodopa). These discrepancies could be explained by the fact that the effect of extracellular synaptic dopamine enhancing agent is mediated by activation of both D1R and D2R and there would also be possible interaction between these receptors and down ward signaling mechanisms. However, the effects of agents agonize or antagonize dopamine receptors are mediated by activation or blockage of only the respective receptors. In addition, Synaptic extracellular dopamine enhancing agents, could also alter dopamine receptor density in cell membrane after repeated application as shown in a previous study by Voulalas et al. (2011) that a significant portion of D1 receptors are translocated from

detergent-resistant membranes to detergent-soluble membranes and the cytoplasmic fraction following daily dopamine enhancing agent like cocaine administration for seven days. Thus, this could also account for the difference observed.

Most of the dopaminergic agents used in the present study did not have an effect on the overall training but a specific day effect. Session specific differences could be due to variation in optimum range of receptor activation required at different time points. Pre-experience to the task or the training status in a task and treatment are among the factors that could lead to the variation in optimum range of receptor activation in different brain regions.

Consistent with a previous work, the training status in the T-maze could be cited as a reason for session specific differences. D1R agonist infusions into the PFC resulted in a memory strength dependent effect. Stronger memories (short delay) were disrupted, whereas weaker memories (long delay) were improved at all drug concentrations used (Floresco and Phillips, 2001). The functional interplay between different working memory related brain regions and region specific optimal ranges may also contribute to the result of session specific differences of responses to the drug treatment. Especially the communication between the PFC and basal ganglia determine working memory (Gruber et al., 2006; van Schouwenburg et al., 2010).

Differences in the optima of dopamine concentrations and receptor specific transmissions within these areas during training may therefore explain the results (Seamans et al., 1998; Chudasama and Robbins, 2004). Especially day 2 performance was sensitive for the blockade of both D1R and D2R, suggesting that at this training stage a dopaminergic overstimulation impairs working memory. This is further supported by the smaller agonist effect on this day. Therefore,

dopaminergic inhibition at this stage might protect against internal noise (Williams and Castner, 2006; Avery and Krichmar, 2015) and enhance working memory.

The treatment might also change the molecular basis of processing of working memory in dose related manner and could lead to variation in effect at different time points. The possible treatment based changes might be attributed to the propensity of developing tolerance by receptors on treatment with specific agonists. D2 (Goggi et al., 2007) and D1 (Dumartin et al., 2008) receptors are prone to internalization, while D3 (Westrich *et al.,* 2010) and D4 (Spooren *et al.,* 2010) receptors are resistant. Therefore, the dopamine receptor subtype composition may change due to these processes and could contribute to the observed changes in working memory performance. In addition, bidirectional functions of a specific receptor type at different dopamine concentrations as well as synergistic and antagonistic physiological functions of different receptor subtypes has also been reported (Seamans and Yang ; 2004). For example, a non linear inverted-U-shaped dose-response profile of postsynaptic dopamine effects in the PFC has been described for working memory (Seamans and Yang, 2004; Cools and D'Esposito, 2011; Williams and Goldman-Rakic, 1995; Vijayraghavan et al., 2007). Similar dose-dependent effects of D2 receptors on neuronal plasticity have also been described in human motor cortex (Monte-Silva et al., 2009; Fresnoza et al., 2014). Skinbjerg *etal*. (2010) reported a decrease in *in vivo* radio ligand binding to the D2 receptor during PET studies after amphetamine induced increased extracellular dopamine for several hours. Induction of long-term depression in the striatum of mice has been shown to be modulated by D2 receptor affinity (Baca et al., 2013).

In general, the training related changes in neuronal circuits, molecular and dopaminergic processes could time specifically interfere with exogenous interventions of dopaminergic drugs (Klingberg, 2010; Buschkuehl et al., 2012; Söderqvist et al., 2012).

The findings of the present study suggest that optimal range of dopaminergic transmission or receptor activation required to modulate spatial working memory is also task dependent. Water maze working memory task also require D1R activity (Wisman et al., 2008; Xing et al., 2012; Murphy et al., 2015) like T- maze task. However, Comparison of T- maze and water maze tasks using D1R agonist, revealed significant differences between D1R agonist treated and control groups in T-maze and no significant difference was found between treated and control rats in the water maze task. This could be explained as follows. i. The endogenous adaptation of the dopaminergic system to the task might make it less sensitive for external manipulations or exogenous treatment in water maze task. Day to day performance differed between the two tasks. In water maze task trained rats, there was a rapid improvement from day 1 to day 2 in control and low dose group but not high dose treated rats, whereas T-maze treated rats did not show significant differences between days. Thus, the working memory underlying mechanisms (but not that of specific memories) and circuitries may be rapidly strengthened in water maze trained rats making additional activations by the agonist less effective and therefore cause no differences between control and treated animals. Strengthening remains in low dose treated rats at day three, whereas in controls performance at day 3 returns to day 1 levels. Thus, similar to the T-maze day 2 seems to be a crucial sensitive time point for working memory performance. ii. Performance difference between the tasks could be due to the different brain regions and cell types involved. The T- maze task is more stressful for animals (Korz and Frey, 2004). Thus, it is likely that the T-maze in contrast to the water maze task involves the amygdala (Zancada-Menendez et al., 2017). Task

dependent differences could be observed only for the high dose. D1R agonist treated rats exhibiting deterioration of working memory at day 1 and 2 in the water maze compared to the T-maze with the most pronounced effect at day 2, which again point to a time dependent sensitivity and restructuring of the dopaminergic system.

To determine the downward signaling mechanisms following dopamine receptor activation in spatial working memory modulation, cAMP level in the PFC region of the brain was considered. Because most of the previous animal studies (Cai and Arnsten 1997; Uylings et al., 2003; Kellendonk et al., 2006) support that PFC plays a critical role in rodents working memory. In cAMP level measurement, both D1R antagonist (1μg) and D2R agonist (5 μg) exhibited significantly higher levels than vehicle controls. No increment in cAMP measurement for D1R agonist treated rats. The absence of increment in cAMP level in rats treated with D1R agonist could be due to signaling via the Gq pathway (Egorov et al. 2002; Birnbaum et al. 2004; Runyan et al. 2005) or signaling through G protein-independent pathways such as those involving β-arrestin (Beaulieu et al., 2007) which are involved in working memory but without increase in cAMP level.

Furthermore, a reduction in D1 receptor density can be cited as a reason. A previous study by McNab et al. (2009) examined dopamine D1 and D2 receptor density before and after training reported a negative correlation between training-related performance increase and changes in cortical D1 binding potential in prefrontal and parietal cortices, which mainly resulted from a decrease in D1 receptor density. Thus, it appears that reduction of D1 receptor density could possibly why there was no increase in cAMP level from D1R activation. The present data may not be sufficient to explain the underlying mechanisms for increase in cAMP level observed in D1R

antagonist and D2R agonist treated rats but very likely intracellularly, the actions of dopamine receptors (Vijayraghavan et al., 2007) and learning related modulations induced by alpha-2A (Wang et al., 2007), and beta1 receptors may converge on the cAMP signaling pathway ((Mochida et al., 1987; Ramos et al., 2008, Gamo and Arnsten, 2011).

6. Conclusions

The present study shows that trainable working memory can be modulated by extracellular dopamine-increasing compounds in precise time windows and partly independent of the substance used. D1R mechanisms appear to be the main modulator of spatial working memory, whereas D2R activity is less involved. This study also supports the hypothesis that working memory is modulated by optimal dopaminergic transmission, and suggests that these optimal ranges can change during the training due to the changed pre-experience probably at the cognitive, molecular and physiological level not only between tasks but also within tasks.

7. Suggestion for future works

The evaluation of the underlying mechanisms would contribute to the understanding of dopaminergic mechanisms in modulating working memory as well of sub-chronic effects of dopaminergic cognitive enhancers.

More work is needed in future research to apply multiple methods of investigation, that is, to assess behavioral performance and combine those data with imaging data such as fMRI or to apply multiple analytical tools to shed light on the involved brain regions and cell types and their interaction during working memory, to distinguish among several possible neural mechanisms that could account for training. This may contribute to the revelation of dopaminergic modulation of working memory and task dependent optimization of cognitive enhancing pharmacological treatment.

8. References

1. Abi-Dargham, A., and Moore, H. (2003). Prefrontal dopamine transmission at D1 receptors and the pathology of schizophrenia. *Neuroscientist.* 9(5):404-416.
2. Ambree, O., Richter, H., Sachser, N., Lewejohann, L., Dere, E., de Souza Silva M.A., Herring, A., Keyvani, K., Paulus, W., and Schabitz, W.R. (2009) Levodopa ameliorates learning and memory deficits in a murine model of Alzheimer's disease. *Neurobiol. Aging* 30:1192–1204.
3. Andersen, P. H., and Jansen, J. A. (1990). Dopamine receptor agonists: selectivity and dopamine D1 receptor efficacy. *Eur. J. Pharmacol.* 188, 335–347.
4. Arnsten, A.F., Paspalas CD., Gamo, N.J., Yang Y., and Wang, M. (2010) Dynamic network connectivity: A new form of neuroplasticity. *Trends in Cognitive Sciences.*
5. Arnsten, A.F.T., and Goldman-Rakic, P.S. (1998). Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. *Arch Gen Psychiatry* 55:362–368.
6. Aultman, J.M., and Moghaddam, B. (2001). Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinically relevant task, *Psychopharmacology*. 153: 353-364.
7. Avery, M.C., and Krichmar, J.L. (2015). Improper activation of D1 and D2 receptors leads to excess noise in prefrontal cortex, *Front. Comput. Neurosci.* 9-31.
8. Baca, M., Allan, A.M., Partridge, L.D., and Wilson, M.C. (2013). Gene-environment interactions affect long-term depression (LTD) through changes in dopamine receptor affinity in Snap25 deficient mice, *Brain Res.* 1532: 85–98.

9. Baddeley, A.D., Allen, R.J., and Vargha-Khadem, F. (2010). Is the hippocampus necessary for visual and verbal binding in working memory? *Neuropsychologia* 48:1089–95

10. Baddeley, A. D. (1986). Working memory. New York, NY, US: *Clarendon Press/Oxford University Press*.

11. Baddeley, A. D. (1992). Working memory. *Science, 225,* 556-559

12. Baddeley, A. D. (2000). The episodic buffer: A new component of working memory? *Trends in Cognitive Sciences*, *4*, 417–423.

13. Baddeley, A. D. (2002). Is working memory still working? *European Psychologist, 7,* 85–87

14. Baddeley, A. D. (2003). Working memory: Looking back and looking forward. *Nature Reviews Neuroscience, 4,* 829-839.

15. Baddeley, A. D., and Logie, R. H. (1999). The multiple-component model. In A. Miyake & P. Shah (Eds.), Models of working memory: Mechanisms of active maintenance and executive control *New York: Cambridge University Press. 28–61*.

16. Baddeley, A. D., and Hitch, G. J. (1974). Working memory. In G. A. Bower (Ed.), The Psychology of Learning and Motivation, *New York: Academic Press* : 8. 47–89

17. Beato, R., Levy, R., Pillon, B., Vidal, C., du Montcel, S.T., Deweer, B., Bonnet, A.M., Houeto, J.L., Dubois, B., and Cardoso, F. (2008). Working memory in Parkinson's disease patients: clinical features and response to levodopa, *Arq. Neuropsiquiatr.* 66: 147-151.

18. Beaulieu, J.M., Gainetdinov, R. R., and Caron,M.G. (2007).TheAkt-GSK- 3 signaling cascade in the actions of dopamine. *Trends Pharmacol.Sci.* 28: 166–172.

19. Bradley, V. A., Welch, J. L., and Dick, D. J. (1990). Visuospatial working memory in Parkinson's disease. *Journal of Neurology, Neurosurgery and Psychiatry,* 52: 1228-1235.

20. Brozoski, T.J., Brown, R.M., Rosvold, H.E., and Goldman, P.S. (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* 205: 929–932.

21. Bubser, M., and Schmidt, W.J. (1990). 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. *Behav Brain Res* 37: 157–168.

22. Buschkuehl, M., Jaeggi, S.M., and Jonides, J. (2012). Neuronal effects following working memory training, *Dev. Cogn. Neurosci.* 2 (Suppl. 1) 167–179.

23. Bushnell, P.J., and Levin, E.D. (1993). Effects of dopaminergic drugs on working and reference memory in rats, *Pharmacol. Biochem. Behav*. 45:765-776.

24. Cai, JX, and Arnsten, AF. (1997). Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. *J Pharmacol Exp Ther* 283: 183–189.

25. Cassel, J.-C., Pereirade Vasconcelos, A., Loureiro, M., Cholvin, T., Dalrymple- Alford, J.C., and Vertes, R.P. (2013).The reunions and rhomboid nuclei: neuroanatomy, electrophysiological characteristics and behavioral implications. *Prog. Neurobiol.* 111:34–52.

26. Castner, S.A, Williams, G.V., and Goldman-Rakic, P.S. (2000). Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. *Science* 287: 2020–2022.

27. Chudasama, Y., and Robbins, T.W. (2004). Dopaminergic modulation of visual attention and working memory in the rodent prefrontal cortex, *Neuropsychopharmacology* 29:1628-1636.

28. Churchwell, J.C., Morris, A.M., Musso, N.D., and Kesner,R.P.(2010).Prefrontal and hippocampal contributions to encoding and retrieval of spatial memory. *Neurobiol. Learn.Mem.* 93: 415–421.

29. Cohn, J. D., Perlistein W.M., braver, T S, Nystrom, L. E., Noll, D. C., Jonides, J and Smith, E.E.(1997). Temporal dynamics of brain activation during a working memory task. *Nature* 386:604-608.

30. Collins, P., Roberts, A.C., Dias, R., Everitt, B.J., and Robbins, T.W. (1998). Perseveration and strategy in an ovel spatial self-ordered sequencing task for non human primates: effects of excitotoxic lesions and dopamine depletions of the prefrontal cortex. *J. Cogn. Neurosci.* 10: 332–354.

31. Cools, R., and D'Esposito, M. (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control, *Biol. Psychiatry* 69:113-125.

32. Costa, A., Peppe, A., Dell'Agnello, G., Carlesimo, G., Murri, L., Bonuccelli, U., and Caltagirone, C. (2003). Dopaminergic modulation of visuospatial working memory in Parkinson's disease, Dementia *Geriatr. Cogn. Disord.* 15 :55–66.

33. Courtney, S.M., Petit, L., Maisog, J.M., Ungerleider, L.G., and Haxby, J.V. (1998). An area specialized for spatial working memory in human frontal cortex. *Science* 279: 1347–1351

34. Cowan, N. (1999). An embedded-process model of working memory. In A. Miyake & P. Shah (Eds.), Models of working memory: Mechanisms of active maintenance and executive control, Cambridge, England: *Cambridge University Press* 62–101.

35. Cowan, N. (2005). Working memory capacity. New York, NY: *Psychology Press*.

36. Dalley, J.W., Cardinal, R.N., and Robbins, T.W. (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates *Neuroscience and Biobehavioral Reviews* 28: 771–784

37. Deguchi, Y., Donato, F., Galimberti, I., Cabuy, E., and Caroni, P. (2011). Temporally matched subpopulations of selectively interconnected principal neurons in the hippocampus. *Nature neuroscience* 14:495-504.

38. Dehn, M.J. (2008). Working memory and academic learning: Assessment and intervention. *New Jersey: John Wiley and Sons.*

39. Di Pietro, N.C., Black, Y.D., Green-Jordan, K., Eichenbaum, H.B., and Kantak, K.M. (2004). Complementary tasks to measure working memory in distinct prefrontal cortex subregions in rats. *Behav Neurosci* 118: 1042–1051.

40. Dudchenko, P.A. (2004). An overview of the tasks used to test working memory in rodents, *Neurosci. Biobehav. Rev.* 28: 699–709

41. Dumartin, B., Caillé, I., Gonon, F., and Bloch, B. (1998). Internalization of D1 dopamine receptor in striatal neurons in vivo as evidence of activation by dopamine agonists, *J. Neurosci.* 18 :1650-1661.

42. Duvernoy, H. M. (2005). The human hippocampus: functional anatomy, vascularization, and serial sections with MRI (3rd ed.*). Berlin*; *New York: Springer.*

43. Engle, R.W., Kane, M.J., and Tuholski, S.W. (1999). Individual differences in working memory capacity and what they tell us about controlled attention, general fluid intelligence, and functions of the prefrontal cortex. In *Models of Working Memory: Mechanisms of Active Maintenance and Executive Control*, ed. A Miyake, P Shah, *New York: Cambridge Univ. Press:* 102–34.

44. Engle, R.W., and Kane, M.J. (2004). Executive attention, working memory capacity and two-factor theory of cognitive control. In The Psychology of Learning and Motivation, ed. B Ross, *New York: Elsevier*. pp. 145–99.

45. Engle, R.W. (2001). What is working memory capacity? In: H.L. Roediger III, J.S. Nairne, I. Heath & A.M. Suprenant (eds.), and the nature of remembering: Essays in honor of Robert G. Crowder Washington, D.C.: *American Psychological Association.* 297-314.

46. Euston, D.R., Gruber,A.J., and McNaughton, B.L.(2012). The role of medial prefrontal cortex in memory and decision making. *Neuron* 76: 1057–1070.

47. Feenstra, M.G., Sumners, C., Goedemoed, J.H., de Vries J.B., Rollema, H., and Horn. A.S. (1983).A comparison of the potencies of various dopamine receptor agonists in models for pre- and postsynaptic receptor activity. *Naunyn Schmiedebergs Arch.Pharmacol*.; 324:108–115.

48. Ferino, F., Thierry, A.M., and Glowinski, J. (1987). Anatomical and elctrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. *Exp. Brain Res.* 65: 421–426.

49. Floresco, S.B., Seamans J.K., and Phillips, A.G. (1997) Selective roles for hippocampal, prefrontal cortical and ventral striatal circuits in radial-arm maze tasks with or without a delay. *J neuroscience* 17:1880-1890.

50. Floresco, S.B., and Phillips, A.G. (2001). Delay-dependent modulation of memory retrieval by infusion of a dopamine D1 agonist into the rat medial prefrontal cortex, *Behav. Neurosci.* 115: 934-939.

51. Floresco, S.B., and Magyar,O. (2006). Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology* 188,567– 585.

52. Fournet, N., Moreaud, O., Roulin, J.L., Naegele, B., and Pellat, J (2000). Working memory functioning in medicated Parkinson's disease patients and the effect of withdrawal of dopaminergic medication, *Neuropsychology* 14: 247–253.

53. Fresnoza, S., Stiksrud, E., Klinker, F., Liebetanz, D., Paulus, W., Kuo, M.F., and Nitsche, M.A. (2014). Dosage-dependent effect of dopamine D2 receptor activation on motor cortex plasticity in humans, *J. Neurosci*. 34: 10701–10709.

54. Funahashi, S., and Kubota, K. (1994). "Working memory and prefrontal cortex." *Neurosci Res* 21(1): 1-11.

55. Funahashi, S., Bruce,C.J., and Goldman-Rakic,P.S.(1993). Dorsolateral prefrontal lesions and oculomotor delayed-response performance:evidence for mnemonic ‘‘scotomas’’. *J. Neurosci.* 13, 1479–1497.

56. Fuster, J.(2015). The PrefrontalCortex. *New York:AcademicPress*

57. Gamo, N.J., and Arnsten, A.F. (2011). Molecular modulation of prefrontal cortex: rational development of treatments for psychiatric disorders. *Behav. Neurosci.* 125:282–296.

58. Goggi, J.L., Sardini, A., Egerton, A., Strange, P.G., and Grasby, P.M. (2007). Agonist-dependent internalization of D2 receptors: imaging quantification by confocal microscopy, *Synapse* 61: 231–241.

59. Goldman,P.S.,and Rosvold, H.E.(1970).Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Exp. Neurol.* 27, 291–304.

60. Granon, S., Passetti F., Thomas, K.L., Dalley, J.W., Everitt, B.J, and Robbins, T.W. (2000). Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J Neurosci* 20: 1208–1215.

61. Griffin, A.L. (2015).Role of the thalamic nucleus reunions in mediating interactions between the hippocampus and medial prefrontal cortex during spatial working memory. *Front. Syst.Neurosci.* 9:29.

62. Gruber, A.J., Dayan, P., Gutkin, B.S., and Solla, S.A. (2006). Dopamine modulation in the basal ganglia locks the gate to working memory, *J. Comput. Neurosci*. 20:153-166.

63. Hampson, R.E., Jarrard, L.E., and Deadwyler, S.A. (1999). Effects of ibotenate hippocampal and extrahippocampal destruction on delayed-match and-nonmatch-to-sample behavior in rats, *J. Neurosci.* 19:1492–1507.

64. Harris, A.Z., and Gordon, J.A. (2015).Long-range neural synchrony in behavior. *Annu. Rev.Neurosci.* 38:171–194.

65. Heidbreder, C.A., and Groenewegen, H.J. (2003). The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neurosci Biobehav Rev* 27:555–579.

66. Hembrook, J.R., Onos,K.D.,and Mair,R.G.(2012).Inactivation of ventral midline thalamus produces selective spatial delayed conditional discrimination impairment in the rat. *Hippocampus* 22: 853–860.

67. Hokfelt, T., Johansson, O., Fuxe, K., Goldstein, M., and Park, D. (1977) Immuno histochemical studies on the localization and distribution of monoamine neuron systems in the rat brain II. Tyrosine hydroxylase in the telencephalon. *Med Biol* **55**:21-40.

68. Honig, W.K. (1978). Studies of working memory in the pigeon. In: Honig WK, editor. Cognitive processes in animal behavior. *Hillsdale: Lawrence Erlbaum;* 211–48.

69. Hoover, W. B., and Vertes, R. P. (2012). Collateral projections from nucleus reuniens of thalamus to hippocampus and medial prefrontal cortex in the rat: a single and double retrograde fluorescent labeling study. *Brain Struct. Funct.* 217:191–209

70. Hoover, W.B., and Vertes, R.P (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct.Funct.* 212:149–179.

71. Hyman, J.M., Zilli, E.A., Paley, A.M., and Hasselmo, M.E. (2005). Medial prefrontal cortex cells show dynamic modulation with the hippocampal theta rhythm dependent on behavior. *Hippocampus* 15: 739–749.

72. Jacobsen, C. F. (1935). Functions of frontal association area in primates. *Arch Neurol Psychiatry* 33: 558-569.

73. Jones, M.W., and Wilson, M.A.(2005).Phase precession of medial prefrontal cortical activity relative to the hippocampal theta rhythm. *Hippocampus* 15:867–873.

74. Kandel, E. R. (Ed.). (2013). Principles of neural science (5th ed.). *New York: McGraw-Hill.*

75. Kane, M.J., Bleckley, M.K., Conway, A.R.A., and Engle, R.W. (2001). A controlled-attention view of working-memory capacity. *J Exp Psychol Gen*, 130:169–183.

76. Kellendonk, C., Simpson, E.H., Polan, J., Malleret, G., Vronskaya, S., Winiger, V., Moore, H., and Kandel, E.R.(2006). Transient and selective over expression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex function. *Neuron* 49: 603–615.

77. Kesner, R.P., Gilbert, P.E., and Lee, I. (2002) Subregional analysis of hippocampal function in the rat. *Neuropsychology of memory* 395-411.

78. Klingberg, T. (2010). Training and plasticity of working memory, *Trends Cogn.Sci.* 14 317-324.

79. Köhler, C., Ericson, H., and Radesäter, A. C. (1991). Different laminar distributions of dopamine D1 and D2 receptors in the rat hippocampal region. Neurosci. Lett. 126, 107–109.

80. Kolb, B., Nonneman, A.J., and Singh, R.K.(1974).Double dissociation of spatial impairments and perseveration following selective prefrontal lesions in rats. *J. Comp. Physiol. Psychol.* 87, 772–780.

81. Korz, V., and Frey, J. U. (2004). Emotional and cognitive reinforcement of rat hippocampal long-term potentiation by different learning paradigms. *Neuron Glia Biol*. 1, 253–261.

82. Kubota, K. and Niki, H. (1971) Prefrontal cortical unit activity and delayed alternation performance in monkeys. *J. Neurophysiol.* 34: 337–347.

83. Lange, K.W., Paul, G.M., Naumann, M., and Gsell, W. (1995). Dopaminergic effects on cognitive performance in patients with Parkinson's disease, *J. Neural Transm.*46: 423–432.

84. Laroche, S., Davis, S., and Jay, T.M. (2000). Plasticity at hippocampal to prefrontal cortex synapses: Dual roles in working memory and consolidation. *Hippocampus* 10: 438–446.

85. Lee, J., and Park, S. (2004). Working memory abnormalities in schizophrenia: A meta-analysis. *J Abnormal Psychol*, in press.

86. Lee, I., and Kesner, R. P. (2003). Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. *J Neurosci* 23(4): 1517-1523.

87. Leonard, S.K., Anderson, C.M., Lachowicz, J.E., Schulz, D.W., Kilts, C.D., Mailman, R.B. (2003). Amygdaloid D1 receptors are not linked to stimulation of adenylate cyclase. *Synapse.* 50:320–333.

88. Luciana, M., Depue, R. A., Arbisi, P., and Leon, A. (1992). Facilitation of working memory in humans by a D-sub-2 dopamine receptor agonist. *J Cogn Neurosci 4*(1):58-68.

89. Mailman, R.B., Schulz, D.W., Kilts, C.D., Lewis, M.H., Rollema, H., and Wyrick, S. (1986). Multiple forms of the D1 dopamine receptor: its linkage to adenylate cyclase and psychopharmacological effects. *Psychopharmacol.Bull.* 22:593–598.

90. Mattay, V.S., Callicott, J.H., Bertolino, A., Heaton, I., Frank, J.A., and Coppola, R. (2000). Effects of dextroamphetamine on cognitive performance and cortical activation. *Neuroimage* 12: 268–275.

91. McCall, R. B., Lookingland, K. J., Bédard, P. J., and Huff, R. M. (2005). Sumanirole, a highly dopamine D2-selective receptor agonist: in vitro and in vivo pharmacological characterization and efficacy in animal models of Parkinson's disease. *J. Pharmacol. Exp. Ther.* 314, 1248–1256.

92. Melis. M.R. and Argiolas, A. (1995). Dopamine and sexual behavior. *Neurosci Biobehav Rev* **19**:19-38.

93. Miller, G.A. (1956). The magical number seven, plus or minus two: some limits on our capacity for processing information. *Psychological review* 63:81.

94. Milner, B., Squire, L.R., and Kandel, E.R. (1998). Cognitive neuroscience and the study of memory. *Neuron* 20:445-468.

95. Mochida, S., Kobayashi, H., and Libet, B. (1987). Stimulation of adenylate cyclase in relation to dopamine-induced long-term enhancement (LTE) of muscarinic depolarization in the rabbit superior cervical ganglion, *J. Neurosci.* 7: 311-318.

96. Monte-Silva, K., Kuo, M.F., Thirugnanasambandam, N., Liebetanz, D., Paulus W., and Nitsche, M.A (2009). Dose-dependent inverted U-shaped effect of dopamine (D2-like) receptor activation on focal and non focal plasticity in humans, *J.Neurosci.* 29:6124–6131.

97. Moser, E.I., Witter, M.P., and Moser, M.B.(2010).‘‘Entorhinalcortex,’’in *Handbook of Brain Microcircuits, eds G.M. ShepherdandS .Grillner (Oxford:Oxford UniversityPress),* 175–190.

98. Murphy, B.L., Arnsten, A.F.T., Goldman-Rakic, P.S., and Roth, R.H. (1996). Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys, *Proc. Natl. Acad. Sci.* USA 93: 1325–1329.

99. Murphy, B.L., Arnsten, A. F., Goldman-Rakic, P.S., and Roth, R.H. (1996). Increased dopamine turn over in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proc.Natl.Acad.Sci.* 93, 1325–1329

100. Murphy, H. M., Ekstrand, D., Tarchick, M., and Wideman, C. H. (2015). Modafinil as a cognitive enhancer of spatial working memory in rats. *Physiol. Behav.* 142,126–130.

101. Nadel, L., and Hardt, T.(2010) Update on memory systems and processes. *Neuropsychopharmacology* 36:251-273.

102. Nadel, L., and Moscovitch, M. (1998). Hippocampal contributions to cortical plasticity. *Neuropharmacology* 37:431-439.

103. Oberauer, K. (2002). Access to information in working memory: Exploring the focus of attention. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *28,* 411–421.

104. Okada, K., and Okaichi, H. (2009). Functional differentiation and cooperation among the hippocampal subregions in rats to effect spatial memory processes. *Behav. Brain Res.* 200:181-191.

105. Okubo, Y., Suhara, T., Zusuki, K., Kobayashi, K., Inoue, O., Terasaki, Y., and Toru M. (1997). Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature,* 385, 634-636.

106. Olton, D.S. (1979) Mazes, maps, and memory. *American Psychologist* 34:583.

107. Packard, M. G., and White, N. M. (1989). Memory facilitation produced by dopamine agonists: role of receptor subtype and mnemonic requirements. *Pharmacol, Biochem & Behav.* 33(3):511-518.

108. Petrides, M. (1996). "Specialized systems for the processing of mnemonic information within the primate frontal cortex." *Philos Trans R Soc Lond B Biol Sci* 351(1346): 1455-1461.

109. Rajasethupathy, P., Sankaran, S., Marshel, J.H., Kim, C.K., Ferenczi, E., and Lee, S.Y (2015).Projections from neocortex mediate top-down control of memory retrieval. *Nature* 526: 653–659.

110. Ramos, B.P., Colgan, L.A., Nou, E., and Arnsten, A.F.T. (2008). β2 adrenergic agonist, clenbuterol, enhances working memory performance in aging animals. *Neurobiolo. aging*. 29(7):1060-1069.

111. Rashid, A.J., So, C.H., Kong, M.M.C., Furtak, T., El-Ghundi, M., Cheng, R., O'Dowd B.F., and George, S.R. (2007). D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc Natl Acad Sci* . 104(2):654–659.

112. Robbins,T.W. (2000). Chemical neuromodulation of frontal-executive functions in humans and other animals. *Exp.Brain Res.* 133: 130–138

113. Rolls, E.T., and Kesner, R.P. (2006) A computational theory of hippocampal function, and empirical tests of the theory. *Progress in neurobiology.* 79:1-48.

114. Ruocco, L.A., Treno, C., Gironi, U.A. Canevale, Arra, C., Mattern, C., Huston, J.P, de Seuza Silva, M.A., Nikolaus, S., Scorziello, A., Nieddu, M., Boatto, G., Illiano, P., Pagano, C., Tino, A., and Sadile, A.G (2014). Prepuberal intranasal dopamine treatment in an animal model of ADHD ameliorates deficient spatial attention, workingmemory, amino acid transmitters and synaptic markers in prefrontal cortex,ventral and dorsal striatum, *Amino Acids* 46 :2105–2122.

115. Russell, V., De Villiers, A., Sagvolden, T., Lamm, M., and Taljaard, J. (1995). Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate putamen of an animal model of attention-deficit hyperactivity disorder – The spontaneously hypertensive rats. *Brain Res*, 676, 343-351.

116. Rusu, G., Nechifor, M., Lupusoru, C.E., and Tartau, L. (2013). Effects of some dopamine agents on modulation of memory processes performance in rats. *Rev Med Chir Soc Med Na Iasi* 117(4):959-64.

117. Rusu, G., Popa, G., Ochiuz, L., Nechifor, M., and Tartau, L. (2014). Effects of some dopamine antagonists on spatial memory performance in rats--experimental research, *Rev. Med. Chir. Soc. Med. Nat. Iasi.* 118:116-124.

118. Santana, N., Mengod, G., and Artigas, F. (2009). Quantitative analysis of the expression of dopamine D1 and D2 receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb. Cortex* 19, 849–860.

119. Sawaguchi, T., Matsumura, M. and Kubota, K. (1988). Dopamine enhances the neuronal activity of spatial short-term memory task in the primate prefrontal cortex. *Neurosci. Res.* 5: 465–473,

120. Sawaguchi, T., and Goldman-Rakic,P.S. (1991).D1dopamine receptors in prefrontal cortex: involvement in working memory. *Science* 22: 947–950.

121. Sawaguchi, T., and Goldman-Rakic, P.S. (1994).The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists in to the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J. Neurophysiol.* 71: 515–528.

122. Saxe, M., Malleret, G., Vronskaya, S., Mendez, I., Sofroniew, M.V., Kandel, E.R., and Hen, R. (2007) Paradoxical influence of hippocampal neurogenesis on working memory. *Proc Natl Acad Sci U S A* 104:4642-4646

123. Seamans, J.K., Floresco, S.B., and Phillips, A.G. (1998). D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat, *J. Neurosci.* 18: 1613-21.

124. Seamans, J.K., and Yang, C.R. (2004). The principal features and mechanisms of dopamine modulation in the prefrontal cortex, *Prog.Neurobiol*. 74: 1-58.

125. Seeman, P., and Madras, B.K. (1998). Anti-hyperactivity medication: methylphenidate and amphetamine. *Mol Psychiatr*3:386–396

126. Siegel, G.J., Albers, R.W., Brady, S.T., and Price, D.L. (2006). Basic Neurochemistry. Burlington, MA: Elsevier Academic Press.

127. Sigurdsson, T. (2015).Neural circuit dysfunction in schizophrenia: insights from animal models. *Neuroscience.*

128. Simon, H.(1981).Dopaminergic A10 neurons and the frontal system. *J. Physiol.* 77:81–95.

129. Skinbjerg, M., Liow, J.S., Seneca, N., Hong, J., Lu, S., Thorsell, A., Heilig, M., Pike, V.W., Halldin, C., Sibley, D.R., and Innis, R.B. (2010). D2 dopamine receptor internalization prolongs the decrease of radioligand binding after amphetamine: a PET study in a receptor internalization-deficient mouse model, *Neuroimage.* 50:1402–1407.

130. Söderqvist, S., Bergman, Nutley, S., Peyrard-Janvid, M., Matsson, H., Humphreys, K., Kere, J., and Klingberg, T. (2012). Dopamine, working memory, and training induced plasticity: implications for developmental research, *Dev. Psychol*. 48 836–843.

131. Spellman, T., Rigotti, M., Ahmari, S.E., Fusi,S., Gogos ,J.A., and Gordon, J.A. (2015). Hippocampal-prefrontal input supports spatial encodingin working memory. *Nature* 52: 309–314.

132. Spooren, A., Rondou, P., Debowska, K., Lintermans, B., Vermeulen, L., Samyn, B., Skieterska, K., Debyser, G., Devreese, B., Vanhoenacker, P., Wojda, U., Haegeman G, and Van Craenenbroeck, K. (2010). Resistance of the dopamine D4 receptor to agonist-induced internalization and degradation, *Cell. Signal.* 22: 600–609.

133. Squire, L.R., Bloom, F.E., McConnell, S.K., Roberts, J.L., Spitzer, N.C., and Zigmond, M.J. (2003). Fundamental Neuroscience. San Diego, CA: *Elsevier Academic Press.*

134. Stahl, S.M. (2000). Essential psychopharmacology: neuroscientific basis and practical applications, 2nd Edition. *Cambridge, UK: Cambridge University Press.*

135. Stam, C.J., de Bruin, J.P., van Haelst, A.M., van der Gugten, J, and Kalsbeek, A. (1989). Influence of the mesocortical dopaminergic system on activity, food hoarding, social-agonistic behavior, and spatial delayed alternation in male rats. *Behav Neurosci* 103:24–35

136. Stubley-Weatherly, L., Harding, J.W., Wright, J.W. (1996). Effects of discrete kainic acid-induced hippocampal lesions on spatial and contextual learning and memory in rats. *Brain res* 716:29-38.

137. Suzuki, W. A., and Amaral, D. G. (1994).Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. *J. Neurosci.*, *14*(3): 1856–1877.

138. Takahashi, H., et al. (2007). Memory and frontal lobe functions; possible relations with dopamine D2 receptors in the hippocampus. Neuroimage 34(4):1643–1649.

139. Takahashi H, et al. (2008). Differential contributions of prefrontal and hippocampal dopamine D(1) and D(2) receptors in human cognitive functions. J Neurosci 28(46): 12032–12038.

140. Thierry, A.M., Gioanni, Y., Dégénétais, E., and Glowinski, J. (2000). Hippocampo-prefrontal cortex pathway:anatomical and electrophysiological characteristics. *Hippocampus* 10:411–419.

141. Tolman, E.C. (1925). Purpose and cognition: The determiners of animal learning. *Psychol. Rev.* 32:285-297.

142. Trossbach, S.V., de Souza Silva, M.A., Huston, J.P., Korth, C., and Mattern, C. (2014). Intranasal dopamine treatment reinstates object-place memory in aged rats, *Neurobiol.Learn. Mem*. 114: 231–235.

143. Ungerstedt, U. (1976) 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine pathway: the turning syndrome. *Pharmacol Ther* **2**:37-40.

144. Uylings, H.B.M., Groenewegen, H.J., and Kolb, B.(2003).Do rats have a prefrontal cortex? *Behav. Brain Res.* 146: 3–17.

145. Uylings, H.B.M., and van Eden, C.G. (1990). Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. *Prog Brain Res* 85:31–62.

146. Vallone, D., Picetti, R., and Borelli, E. (2000). Structure and function of dopamine receptors. *Neurosci. Biobehav. Rev*. 24, 125–132.

147. Van Schouwenburg, M., Aarts, E., and Cools, R. (2010). Dopaminergic modulation of cognitive control: distinct roles for the prefrontal cortex and the basal ganglia *Curr. Pharm. Des.* 16 :2026-2032.

148. Varela, C., Kumar,S., Yang, J.Y., and Wilson, M.A. (2014).Anatomical substrates for direct interactions between hippocampus, medial prefrontal cortex and the thalamic nucleus reuniens. *Brain Struct.Funct.* 219, 911–929.

149. Vertes, R.P. (2006).Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive eprocessing in the rat. *Neuroscience* 142: 1–20.

150. Vijayraghavan, S., Wang, M., Birnbaum, S.G., Williams, G.V., and Arnsten, A.F (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory, *Nat. Neurosci.* 10: 376–384.

151. Von Huben, S. N., Davis, S. A., Lay, C. C., Katner, S. N., Crean, R. D., Taffe, M. A. (2006). Differential contributions of dopaminergic D-sub-1- and D-sub-2-like receptors to cognitive function in rhesus monkeys. *Psychopharmacology.* 188(4):586-596.

152. Voulalas, P. J., Schetz, J., and Undieh, A. S. (2011). Different subcellular distribution of rat brain dopamine receptors and subtype-specific redistribution induced by cocaine. *Mol. Cell. Neurosci*. 46, 645–654.

153. Wang, G.W. and Cai, J.X. (2006). Disconnection of the hippocampal–prefrontal cortical circuits impairs spatial working memory performance in rats. *Behav. Brain Res.* 175**:** 329–336.

154. Wang, H.Y., Undie, A.S., and Friedman, E. (1995). Evidence for the coupling of Gq protein to D1-like dopamine sites in rat striatum: Possible role in dopamine-mediated inositol phosphate formation. *Mol. Pharmacol.* 48: 988–994.

155. Wang, M., Ramos, B.P., Paspalas, C.D., Shu, Y., Simen, A., Duque, A., Vijayraghavan, S., Brennan, A., Dudley, A., and Nou, E. (2007). Alpha 2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. *Cell* 129: 397–410.

156. Weiner, R. I. and Ganong, W. F. (1978). Role of brain monoamines and histamine in regulation of anterior pituitary secretion. *Physiol Rev* 58(4): 905-76.

157. Westrich, L., Gil-Mast, S., Kortagere, S., and Kuzhikandathil, E.V. (2010). Development of tolerance in D3 dopamine receptor signaling is accompanied by distinct changes in receptor conformation, *Biochem. Pharmacol.* 79: 897–907.

158. Wilkerson, A., and Levin, E.D (1999). Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats, *Neuroscience* 3: 743–749.

159. Williams, G.V., and Castner, S.A. (2006). Under the curve: critical issues for elucidating D1 receptor function in working memory, *Neuroscience* 139: 263-276.

160. Williams, G.V., and Goldman-Rakic, P.S. (1995). Modulation of memory fields by dopamine D1 receptors in prefrontal cortex, *Nature*. 376:572-575.

161. Wisman, L. A., Sahin, G., Maingay, M., Leanza, G., and Kirik, D. (2008). Functional convergence of dopaminergic and cholinergic input is critical for hippocampus-dependent working memory. *J. Neurosci.* 28, 7797–7807.

162. Witter, M., and Amaral, D. (2004). The hippocampal region. The rat nervous system 637-703.

163. Wright, A. A. (2007). An experimental analysis of memory processing. *Journal of the Experimental Analysis of Behavior*, *88*(3), 405-433.

164. Xing, B., Guo, J., Meng, X., Wie, S. G., and Lis, S. B. (2012). The dopamine D1 but not D3 receptor plays a fundamental role in spatial working memory and BDNF expression in prefrontal cortex of mice. *Behav. Brain Res.* 235, 36–41.

165. Yang, S.T., Shi, Y., Wang, Q., Peng, J.Y., and Li, B.M.(2014). Neuronal representation of working memory in the medial prefrontal cortex of rats. *Mol. Brain* 7:61.

166. Yoon, T., Okada, J., Jung, M.W., and Kim, J.J. (2008). Prefrontal cortex and hippocampus subserve different components of working memory in rats, *Learn. Mem.* 1597–105.

167. Yu, J.Y., and Frank, L.M. (2015). Hippocampal-cortical interaction in decision making. *Neurobiol. Learn.Mem.* 117: 34–41.

168. Zahrt, J., Taylor, J.R., Mathew, R.G., and Arnsten, A.F. (1997). Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance, *J. Neurosci.* 17: 8528–8535.

169. Zancada-Menendez, C., Alvarez-Suarez, P., Sampedro-Piquero, P., Cuesta, M., and Begega, A. (2017). Requiring collaboration: hippocampal-prefrontal networks needed in spatial working memory and ageing. A multivariate analysis approach. *Neurobiol. Learn. Mem.* 140, 33–42.