Behavioural Brain Research 312 (2016) 9–13

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Repeated application of Modafinil and Levodopa reveals a drug-independent precise timing of spatial working memory modulation

M. Bezu[a], B. Shanmugasundaram[a], G. Lubec[b,*], V. Korz[a,**]

[a] Department of Pediatrics, Medical University of Vienna, Vienna, Austria
[b] Department of Pharmaceutical Chemistry, Faculty of Life Sciences, University of Vienna, Althanstraße 14, Vienna A-1090, Austria

HIGHLIGHTS

- Modafinil and Levodopa modulate spatial working memory time specifically.
- The time window is drug independent.
- Drugs differ in their dose response effects.

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 1 June 2016
Accepted 2 June 2016
Available online 4 June 2016

Keywords:
Dopamine
Working memory
T-maze
Cognition

ABSTRACT

Cognition enhancing drugs often target the dopaminergic system, which is involved in learning and memory, including working memory that in turn involves mainly the prefrontal cortex and the hippocampus. In most animal models for modulations of working memory animals are pre-trained to a certain criterion and treated then acutely to test drugs effects on working memory. Thus, little is known regarding subchronic or chronic application of cognition enhancing drugs and working memory performance. Therefore we trained male rats over six days in a rewarded alternation test in a T-maze. Rats received daily injections of either modafinil or Levodopa (L-Dopa) at a lower and a higher dose 30 min before training. Levodopa but not modafinil increased working memory performance during early training significantly at day 3 when compared to vehicle controls. Both drugs induced dose dependent differences in working memory with significantly better performance at low doses compared to high doses for modafinil, in contrast to L-Dopa where high dose treated rats performed better than low dose rats. Strikingly, these effects appeared only at day 3 for both drugs, followed by a decline in behavioral performance. Thus, a critical drug independent time window for dopaminergic effects upon working memory could be revealed. Evaluating the underlying mechanisms contributes to the understanding of temporal effects of dopamine on working memory performance.

1. Introduction

Working memory has been defined as "central executive" mechanisms' of cognition relating to temporary storage and operation of information in both, humans and animals, in order to guide future response selection [1,2]. Working memory is essential for facilitating complex behaviors. As such, working memory has become a central construct in cognitive neuroscience. Spatial working memory has been considered as a dynamic encoding process of spatial information over a short time, by acquisition and repeatedly updating of changing spatial information over time [2], thus temporally representing a recently visited place to guide forthcoming behavior. The capacity of spatial working memory can therefore be tested by using tasks with a short delay, such as the delayed alternation task.

Working memory depends on a variety of interconnected brain regions, but most of the research supports the main involvement of the prefrontal cortex (PFC) and the hippocampus [3]. Lesions in the medial PFC (mPFC) and hippocampus result in deficits of working memory in rats [4–6]. The mPFC is the rodent equivalent of the

* Corresponding author.
** Corresponding author at: Department of Pediatrics, Medical University of Vienna, Währinger Gürtel 18, 1090 Vienna, Austria.
E-mail addresses: gert.lubec@univie.ac.at (G. Lubec), volker.korz@meduniwien.ac.at (V. Korz).

http://dx.doi.org/10.1016/j.bbr.2016.06.003
0166-4328/

dorsolateral PFC in primates and human subjects. Humans with lesions, particularly in the dorsolateral PFC or the hippocampus show a severe deficit in spatial working memory [7,8].

Experimental evidence has been raised that various neurotransmitters, particularly dopamine in both of these brain regions regulate working memory [3,9–11]. Moreover, balanced stimulation of PFC dopamine receptors appears to be necessary for optimal working memory performance in rodents and primates. An inverted U-shaped relation, thus deficits in working memory by either elevated or deficient cortical dopaminergic transmission has been observed [12,13]. 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 [14,15]. Levodopa therapy in humans and animal models as well as intranasal dopamine application in had a positive effect by increasing extracellular dopamine levels, not only on related motor dysfunction but also on spatial working and reference memory tasks [15–19], although in some studies no effects of dopaminergic medications on spatial working memory in Parkinsonís disease could be determined [20,21]. 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 L-Dopa and inhibition of the reuptake of endogenous dopamine through modafinil. However, the effects of subchronic treatment of these drugs on the repetitive updating of spatial working memory during training is still unclear. Therefore, we investigated the effects of two dopaminergic transmission targeting drugs (modafinil and L-Dopa) 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 [2]. In this task, the animal has to make an alternating choice response between two maze arms to obtain a reward, guided by discrete or spatial cues, the trials separated by a short delay period demanding working memory abilities.

2. Methods and materials

2.1. Subjects

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: $22 \pm 2\,^{\circ}C$; humidity: $55 \pm 5\%$; 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.

2.2. Apparatus

Working memory tests were carried out in a T- maze made of black acrylic consisting of three arms arranged in a T shape. Each goal arm of the maze was 50 cm long, 10 cm in width and equipped with walls with a height of 25 cm. The central arm was 70 cm long with a 20 cm starting box that could be separated 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 reward was 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, 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 PC.

3. Procedure

3.1. Handling and habituation

A total of 69 rats were included in the experiment. 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 ad libitum. The body weight of the animals was maintained to 85% of free feeding weight by providing them with limited amount of pellet daily.

Animals were habituated to a T-maze until they voluntarily ate a piece of pellet placed at the end of each arm. One food reward was provided to rats in the home cage each day for a few days prior to training in order to acclimate the rat to the reward in a familiar environment. 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.

3.2. Drug administration and training

Two mg or 20 mg/kg of a levodopa and carbidopa (Sigma Aldrich) mixture in a ratio of (4:1) dissolved in saline, or 1 mg and 10 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 intraperitoneally (i.p.) 30 min prior to the start of behavioral testing.

A delayed none matching to place task was performed. Each training session consisted of 10 trials (a forced trial followed by 9 choice 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 an error of working memory. In addition, the working memory index was calculated (correct choices/total trials).