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Original Research Article

Effect of dizocilpine (MK-801) on the working memory of rats on a three-panel runway apparatus

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ABSTRACT

Background: Understanding the processes underlying cognitive functions is a prerequisite to develop strategies for the treatment of cognitive deficits. There is a great need for valid animal models for investigating the cognitive enhancing effects of potential therapeutics. Many studies have investigated animal models of cognitive deficits by using animals treated with compounds that compromise cognitive abilities. Glutamate, an excitatory neurotransmitter and abundantly distributed in the central nervous system is involved in memory processes through N-methyl-d-aspartate (NMDA) receptors. The behavioural consequences of blocking the NMDA receptor provide the rationale for cognitive impairment as an animal model for the cognitive deficits associated with dementia. Authors investigated the effect of dizocilpine (MK-801), an NMDA-receptor antagonist (non-competitive) on the working memory in rats using the three-panel runway apparatus.

Methods: Total 24 trained male albino rats were randomly divided into 4 groups of 6 animals each. Varying doses of MK-801 were administered to the animals. Working memory errors and latency periods were evaluated on the three panel Runway apparatus.

Results: Treatment with MK-801 at the dose of 0.03mg/ kg did not result in any significant change in working memory errors or latency period in comparison to saline control. MK-801 treatment at dose of 0.1mg/kg and 0.3mg/kg resulted in a significant increase in the number of working memory errors and latency period as compared to control.

Conclusions: Authors conclude that MK-801 treatment in the dose of 0.1mg/ kg and 0.3mg/kg resulted in working memory deficits on the three-panel runway apparatus. Rats with cognitive deficits induced by the prototypical N-methyl-d-aspartate (NMDA) receptor antagonist MK-801 may provide a relevant animal model of dementia based on the mechanistic approach of blocking NMDA/glutamatergic signalling.

Keywords: Three-panel runway apparatus, MK-801, Working memory

INTRODUCTION

Impairment of cognitive functions is seen in a wide variety of neurodegenerative disorders.[1] Working memory is a form of short-term memory with a limited capacity and an extremely rapid decay.[2] Its impairment is more depictive of memory disorder in the Alzheimer's dementia.[2] A great deal of cognitive research has been performed during the past few decades to understand the synaptic events, synaptoneural connectivity and the cellular mechanisms responsible for working memory. This had been done with the help of various tools, such as, in-vitro tests, in-vivo animal models and behavioral paradigm.[3] Impairment of memory in rats induced by scopolamine is an extensively studied model.[4-6] Various other pharmacological agents have also been used to induce memory impairment in animal models.[1]

Electrophysiological studies indicate the involvement of NMDA receptor in the induction of long-term potentiation (LTP), a form of synaptic plasticity that is involved in memory formation.[7] It has been suggested that learning and memory deficits produced by NMDA receptor antagonists may be related to the blockade of LTP.[7] MK-801 is one of the most studied, uncompetitive NMDA receptor antagonists and could be considered as a reference molecule.[8] Administration of MK-801 in rodents has been demonstrated to block LTP and subsequent cognitive impairment8, disruption of passive avoidance responses and impairment of spatial memory in Morris-water maze and eight-arm radial maze.[9-11]

MK-801 administration in rodents has also been reported to disrupt the working memory in radial-arm maze and Y-maze spontaneous alternation test.[12,13] Furuya et al, designed a three-panel runway apparatus to study the working memory in rats.[5] The model is validated to evaluate cholinergic dysfunction in the hippocampus and is considered to be a superior discriminatory paradigm. However, Glutamatergic dysfunction has never been investigated on the three-panel runway apparatus. Therefore, in the present study we evaluated the acute effect of MK-801, a non-competitive NMDA receptor channel blocker, on the working memory of rats using the three-panel runway apparatus.

METHODS

Animals

Twenty-four male albino rats of wistar strain weighing 150-200gms were used. The rats were provided commercial food pellet and water ad libitum. Animals were housed in groups of 3-4 per cage and kept under controlled room temperature (24±2ºC) in a 12-hour light dark cycle. All experiments were conducted between 0900 and 1600 h in a noise free environment. The study was done at Department of Pharmacology, Lady Hardinge Medical College, New Delhi.

The study period including the training of rats, construction and validation of the three-panel runway apparatus took nine months. The procedures in this study were performed in accordance with the present CPCSEA Guidelines for the Care and Use of Laboratory Animals. The protocol of the study was approved by the Animal Ethics Committee of the Institution and all efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Apparatus

Working memory was assessed with a three-panel runway apparatus (Figure 1).[5,6] In brief, this apparatus has a start box, a goal box and 4 consecutive intervening choice points. Each choice point consisted of 3 panels or gates. The rats were prevented from passing through two of the three panels or gates, by front stoppers and also prevented from returning to the start box or to the previous choice point, by the one-way opening hinged panel gate (Figure 2). When the rats reached the goal box, they received two food pallets, of about 50mg each.

Acquisition training

Initially all the front stoppers were removed so that a rat could pass through any of the 3 panel gates at each choice point. The rats were made to run the task repeatedly until the time that elapsed from leaving the start box to reaching the goal box (latency period) was consistently below 30 sec. Once this time was reached, the rats were given 1 session of 6 consecutive trials per day, with inter-trial period of 2 minutes.

Each day, the sequence of correct panel gate position (open gate) for each rat, was changed according to the sequence chart as originally described by Furuya et al, (Table 1).[5] The number of times an animal pushed an incorrect panel-gate (errors) and the time required for the animal to reach the goal box (latency period) were recorded in every trial of the session. Errors and latency periods of each of the 6 trials were added to obtain the total number of errors and total latency period of the session. A rat was selected for the experiment if it achieved the criterion of ≤12 mean errors per session in 3 consecutive sessions.

Table 1: Twelve types of sequences of correct panel position in the three-panel runway apparatus.

	Choice Point 1	Choice Point 2	Choice Point 3	Choice Point 4
1.	a→	b→	a→	c→
2.	c→	b→	c→	a→
3.	b→	c→	b→	a→
4.	c→	a→	c→	b→
5.	c→	b→	a→	c→
6.	a→	c→	b→	c→
7.	c→	a→	b→	a→
8.	b→	a→	c→	b→
9.	b→	c→	a→	c→
10.	a→	b→	c→	b→
11.	b→	a→	c→	a→
12.	a→	c→	b→	a→

Study design

Trained and selected rats were randomly divided into 4 groups (6 rats per group). MK-801 (Merck Research Laboratories, USA) was dissolved in saline and was administered intraperitoneally (ip) in three doses of 0.03mg/kg, 0.1mg/kg and 0.3mg/kg, 30 minutes before the session, on the test day to group II, III and IV respectively. Saline was administered ip to the control animals (group I). MK-801 and saline were administered at a volume of 0.1ml/ 100g-body weight.

Statistical analysis

Working memory errors and latency period per trials and session were presented separately and expressed as Mean±SEM. The comparison of difference in working memory errors and latency periods (expressed as Mean±SEM) between different groups was determined with a one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test when F-ratios reached significance ($p < 0.05$).

RESULTS

Acquisition training

A total of 30 rats were trained in around 15 training sessions, of which 6 rats failed to achieve the selected criteria of ≤12 mean working memory errors per session. A total of 24 rats achieved the selected criteria of ≤12 mean working memory errors per session in the three-panel runway apparatus.

The acquisition training of the rats took 4 months. After the training period, the selected rats achieved ≤12 mean working memory errors per session and the average latency period of a single trial of a session was consistently below 30 sec.

Working memory errors

MK-801 treatment in the dose of 0.03mg/kg did not result in any significant increase in the total number of working memory errors in the session (11.33±0.96) as compared to the Saline control group. The dose of 0.1mg/kg resulted in a significant increase in the total working memory errors in the session (18.00±1.53), in comparison to the saline control group ($P < 0.001$, Table 2). Likewise, the dose of 0.3mg/kg resulted in a significant increase in the total working memory errors in the session (19.50±0.96), in comparison to the saline control group ($P < 0.001$, Table 2). The mean working memory errors in the first trial remained constant at approximately 4 in all the groups (Table 2).

Latency period

MK-801 administration in the dose of 0.03mg/kg did not result in any significant increase in the total latency period of the session as compared to the saline control group. The dose of 0.1mg/kg resulted in a significant increase in the total latency period of the session (115.14±9.28) as compared to the saline group ($P < 0.05$, Table 2). The dose of 0.3mg/kg resulted in a significant increase in the total latency period of the session (132.34±4.25) as compared to the saline group ($P < 0.001$, Table 2).

Table 2: Effect of MK-801on the first trial and total number of working memory errors in rats in a session and the total latency period of a session.

Groups	No. of errors in the first trial (Mean±SEM)	Total no. of errors in a session. (Mean±SEM)	Total latency of a session (secs) (Mean±SEM)
Group I- Saline	4.01±0.27	9.00±0.45	57.25±1.31
Group II- MK-801 (0.03mg/kg)	4.50±0.34	11.33±0.96	68.39±4.26
Group III- MK-801 (0.1mg/kg)	5.05±0.37	18.00±1.53[b]	115.14±9.28[a]
Group IV- MK-801 (0.3mg/kg)	5.00±0.26	19.50±0.96[b]	132.34±4.25[b]

The values are Mean±SEM (n = 6 in each group). P values: [a]<0.05, [b]<0.001 versus saline group; One-way ANOVA: $F_{3, 20}$ =12.95; P <0.001 (Errors), $F_{3, 20}$ =8.00; P <0.001 (Latency period), $F_{3, 20}$ =2.39; P <0.001 (first trial errors)

DISCUSSION

The ability to make fine discriminations between complex stimuli that differ only in some subtle manner is regarded as a mark of enhanced cognition status.[14] Delayed matching to sample procedure is a frequently used a discriminatory paradigm for studying working memory in rodents.[14] Working memory allows animals to remember information that is useful for a single session of an experiment but not for subsequent sessions.[15] Three-panel runway apparatus, a modification of Hill's apparatus, is designed to study working memory in rats where more than 2 choices were available for the rat at each choice point and thus is considered to be superior in assessing the discriminatory paradigm.[5]

In the present study, all the rats had acquired a steady state (basal average score of ≤12 errors per session) after repeated acquisition procedure.[5] Repeated acquisition procedure, earlier used with operant apparatus, required 40-60 sessions of training until attaining a steady state.[5] In the present study on three-panel runway apparatus rats could be trained to reach a steady state in about 15 sessions. Thus, completion of learning task is easily attained in this apparatus. The prime determinant of working memory deficits in three-panel runway apparatus is the number of

working memory errors committed by the rodent in a session. Latency period however, is a weak indicator of working memory in this apparatus, as latency period would in turn depend on, the degree of appetite, speed of the rat and more importantly, the number of training sessions before the test run.

Scopolamine, a central anticholinergic agent, induced amnesia in rodents is a widely cited model for human dementia.[3,4] In a previous study scopolamine administration in rats resulted in increased number of errors and latency periods in the three-panel runway apparatus.[5,6,16] In the present study, MK-801 in the dose of 0.1mg/kg and 0.3mg/kg resulted in a significant increase in both working memory errors and total latency periods in the three-panel runway apparatus.

MK-801 in the dose of 0.01mg/ kg and 0.1mg/ kg was shown to cause amnesia in passive avoidance responses.[9,17] Spatial memory impairment was seen in rats in the dose of 0.16mg/kg in the eight-arm radial maze and in the dose of 0.1mg/kg in the Morris water maze.[18,19] In the working memory paradigms 0.3 mg/kg dose of MK-801 disrupted the spatial working memory in a delay interposed radial arm maze, while 0.1mg/kg dose disrupted spatial working memory in an Y-maze exploration in rodents.[20,21] A dose of 0.25mg/kg of MK-801 produced somnolence and impaired the rat's ability to swim in the Morris water maze.[10] The above findings of working memory disruptions are in accordance with the findings of our study on the three-panel runway apparatus by MK-801 in the doses of 0.1mg/kg and 0.3mg/kg.

In the present study amnesic doses of MK-801 treatment resulted in no significant increase in the number of errors in the first trial, when compared to the saline treated group. This is in accordance with other studies, where amnesic doses of scopolamine and ischaemia caused no significant increase in the first trial error responses in comparison to the saline group.[5,15]

In a study by Filliat et al, a dose of 0.1mg/kg of MK-801 resulted in both significant ataxia and hyperlocomotion, while significant ataxia and hyperlocomotion was noted at 0.2mg/kg and 0.05mg/ kg doses respectively.[18] In another study on rats in eight arm radial maze, MK-801 in the dose of 0.16mg/kg resulted hyperlocomotion and increase in the number of arm entries.[17] In present study, although ataxia was not graded, it was seen in some of the animals at the dose of 0.3mg/kg. Although locomotor activity was not assessed in our study, the increase in the latency periods at doses of 0.1mg/kg and 0.3mg/kg were caused despite locomotion hyperactivity caused by MK-801, probably as a result of more time spent in making working memory errors and spatial memory disruption.[10,13]

CONCLUSION

The present study revealed that MK-801, a non-competitive NMDA receptor channel blocker, in the dose of 0.1mg/kg and 0.3mg/kg induced working memory impairment in the three-panel runway apparatus suggesting a role of glutamatergic neurotransmission in the cognitive processes. MK-801 induced working memory impairment on the three-panel runway apparatus could serve as a model of human dementia.

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