STUDY OF DOSE RESPONSE CHARACTERISTICS OF SUBANAESTHETIC DOSES OF KETAMINE ON COGNITIVE FUNCTION AND ANXIETY BEHAVIOUR IN RATS

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ABSTRACT

Objectives: Ketamine, a Schedule III drug under the Controlled Substances Act, is a dissociative anaesthetic having combination of stimulant, depressant, hallucinogenic, and analgesic properties. The main objective of this study was to assess the dose-response characteristics of subanaesthetic doses of ketamine on cognitive function and anxiety behaviour in rats. Methods: After overnight fasting, Wistar albino rats were subjected to habituation on T maze, forced alternation training and discrete, paired-trials delayed alternation task: each trial-pair consisted of a forced trial and a choice trial. Upon reaching a stable performance level, the animals randomly received a normal saline or ketamine injection (s.c.) 10 min (8mg/kg) and 25 min (12mg/kg) prior to testing. Anxiety analysis was done using elevated plus maze assessing total time spent in the closed arms and open arm, entries into the open and closed arms and number of head dipping. Results: Ketamine at the dose of 8 mg/kg and 12 mg/kg significantly increased the number of errors (2.8±0.9 and 4.6±0.3) on T maze (p<0.001) significantly reducing the percentage of correct responses compared to control (1±0.3). Also, number of entries in open arm and average time spent in open arm and number of head dipping by rats treated with ketamine were significantly higher compared to control (p<0.001). The mechanism by which it produces...
adverse behavioural effects, partly have been attributed to blockade of NMDA receptors located on inhibitory GABAergic neurons. **Conclusion:** Ketamine in subanaesthetic doses impaired working memory deficit assessed by discrete-trial delayed alternation test and showed possible anxiolytic property.

**Keywords:** Ketamine, cognitive function, anxiety behaviour, NMDA receptors, GABAergic neurons.

**INTRODUCTION**

A Schedule III drug under the Controlled Substances Act, Ketamine is a dissociative anaesthetic that has a combination of stimulant, depressant, hallucinogenic, and analgesic properties and is frequently abused because of its psychoactive properties.\(^1\) Ketamine, a phencyclidine hydrochloride (PCP) derivative, is a noncompetitive antagonist of the \(N\)-methyl-D-aspartate (NMDA) subtype of glutamate receptor. NMDA receptor hypofunction in the brain is associated with a range of effects on cognition in animals. For example, NMDA receptor antagonists disrupt memory function in rodents\(^2-6\) and in non-human primates.\(^7\) Other studies have demonstrated that mice lacking the NMDA R1 receptor subunit exhibit spatial memory deficits.\(^8\)

At subanesthetic doses, the role of ketamine, as a noncompetitive NMDA receptor antagonist, in blocking the processing of nociceptive inputs has led to its use in chronic pain syndromes management.\(^9,10\) However, in compulsive users ketamine use was reported to induce cognitive impairments.\(^11\) Therefore, findings and reports suggest the need for further experimental and clinical studies of the role of ketamine intake on the body systems, most especially the brain/behaviour in particular. Main objective of this study was to assess the dose-response characteristics of subanaesthetic doses of ketamine on cognitive function using behavioural measures which are likely to have relevance to clinical symptomatology. Second aim of this study was to investigate effects of ketamine on the anxiety behaviour using elevated plus maze.

**METHODS and MATERIALS**

The rats were procured from Central Animal House facility of Smt. Kashibai Navale Medical College, Pune. They were provided with standard commercial pelleted diet *ad libitum* and Aquaguard drinking water. The training protocol was adapted from Aultman and Moghadam (2001).\(^12\)
**Discrete Paired-Trial Delay T-Maze Task Design:** After overnight fasting, rats were habituated to the T maze with food rewards scattered throughout the maze. This was followed by one week of “forced alternation” training, where rats were allowed to retrieve the reward from only one arm, the other being blocked, ten trials each day in alternating pattern. Following the forced alternation training, the discrete, paired-trials delayed alternation task began. Each trial-pair consisted of a forced trial and a choice trial, separated by a delay interval of 10 s (memory retention). Correct choice was scored if the rat entered the baited arm; entries into the initially visited forced arm were scored as errors. Upon reaching a stable performance level, the animals randomly received a normal saline s.c. or ketamine injection (8 and 12 mg/kg s.c.) at 10 min (8 mg/kg) and 25 min (12 mg/kg) prior to test.

For assessing anxiety, rats were placed in the centre of elevated plus maze to explore for 5 min. Measurements included total time spent in the closed arms as well as, entries into the open and closed arms and head dipping.

**Flow chart of study procedure**

1. Screening of Wistar albino rats
2. Habituation Phase X 3 days
   - (Free exploration on T maze)
3. Forced Alternation Training
   - Ten trials with 10-seconds inter-trial interval
4. Discrete Paired-Trials Delayed Alternation Task
   - Forced trial alternating with Choice Trial by a delay interval of 10 seconds
   - Ten Trial-pairs separated by 30s inter-trial interval
5. GROUP 1
   - n = 6
   - Vehicle (normal saline) s.c
6. GROUP 2
   - n = 6
   - Ketamine 8 mg/kg s.c.
7. GROUP 3
   - n = 6
   - Ketamine 12 mg/kg s.c.

**Assessment of cognition and anxiety parameters on T maze & Elevated Plus maze respectively**

**STATISTICS**

The observations were analysed using Students t test. Number of errors on T maze due to Ketamine (8 mg/kg and 12 mg/kg) were compared with the Control group for Cognitive
effects of subanaesthetic doses of Ketamine. The effects of subanaesthetic doses of Ketamine on Anxiety parameters was found by comparing effects of Ketamine (8 mg/kg and 12 mg/kg) with Control group. Also, intragroup comparison of effects of subanaesthetic doses of Ketamine on Anxiety parameters was done.

RESULTS
As shown in figure 1, Ketamine in the doses of 8 mg/kg and 12 mg/kg significantly increased the number of errors (2.8±0.9 and 4.6±0.3) on T maze (p<0.001) significantly reducing the percentage of correct responses compared to control (1±0.3).

* p<0.05, ** p<0.001, ***p<0.001 vs control.

Figure 1: Effect of subanaesthetic doses of ketamine on cognition

Also, number of entries in open arm and average time spent in open arm and number of head dipping by rats treated with ketamine were significantly higher compared to control (p<0.001) as shown in table 1. On dose comparison, effects of ketamine at the dose of 12 mg/kg on the anxiety parameters were significant compared to 8mg/kg of ketamine.

Table 1: Effects of subanaesthetic doses of ketamine on anxiety

<table>
<thead>
<tr>
<th>Anxiety Parameters</th>
<th>Control</th>
<th>Ketamine (8mg/kg)</th>
<th>Ketamine (12 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Entries in Open Arm</td>
<td>1 ± 0.3</td>
<td>2 ± 0.3**</td>
<td>5 ± 0.3***Ⅰ</td>
</tr>
<tr>
<td>Time Spent in Open Arm</td>
<td>5.2 ± 2.4</td>
<td>178 ± 13.3***</td>
<td>250 ± 10.9***Ⅰ</td>
</tr>
<tr>
<td>Time Spent in Closed Arm</td>
<td>242.5 ± 14.2</td>
<td>19.8 ± 7.8***</td>
<td>16.7 ± 8.8***</td>
</tr>
<tr>
<td>No. Of Head Dipping</td>
<td>0.8 ± 0.4</td>
<td>2.8 ± 0.5**</td>
<td>6 ± 0.8***Ⅰ</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.001, ***p<0.001 vs control, Ⅰ p<0.001 vs Ketamine 8mg/kg, Ⅰ p<0.01 vs Ketamine 8mg/kg
DISCUSSION

Glutamate receptors are important for cognition and the NMDA receptors in particular play a profound role in cognition and synaptic plasticity. NMDA receptors are distributed broadly in the brain and densely within the hippocampus and neocortex. This distribution may underlie the preferential impairment of working and episodic memory after low to moderate doses of NMDA receptors antagonists. Studies in rodents and nonhuman primates demonstrate that NMDA receptor antagonists affect performance in tasks that engage frontal cortex and/or hippocampus.\[13]\n
The mechanism by which ketamine produces its adverse behavioural effects, at least partly, have been attributed to the blockade of NMDA receptors located on inhibitory GABAergic neurons limbic and subcortical brain regions.\[14-16]\ This disinhibitory action leads to increase in the neuronal activity and excessive glutamate and dopamine release in the prefrontal cortex and limbic striatal regions.\[14-19]\n
The discrete-trial delayed alternation task is a working memory-related paradigm. Performance of this task depends on the functional integrity of the prefrontal cortex, and it is sensitive to the acute effects of psychostimulants.\[12]\ As previously reported, ketamine could produce working memory deficit in spatial delayed alternation task at the dose of 20-30 mg/kg but not 10 mg/kg.\[20]\ Here we demonstrated that doses of 8 and 12 mg/kg ketamine are also able to impair working memory. Thus, the discrete-trial delayed alternation task provides more sensitive measurement for the acute effects of psychostimulants on working memory.
which might explain how such a low dose of ketamine was found to impair the percentage of correct responses in this task.

Gabore Imre et al. demonstrated the lack of significant difference in the mean velocity of rats in T maze between the ketamine and saline treated groups which suggests that ketamine-induced impairment of the discrete-trial delayed alternation task cannot be attributed primarily to interference by locomotor activity; rather, a disruption in associative function might have occurred.\textsuperscript{[21]}

**CONCLUSION**

In summary, the present experiments have shown that ketamine in subanesthetic doses cause dose dependent impairment of working memory assessed by discrete-trial delayed alternation test and showed possible anxiolytic property. Therefore NMDA mechanisms antagonized by ketamine appear to be selectively involved in the cognition deficit.

**REFERENCES**


