EFFECTS OF RISPERIDONE ON LEARNING AND MEMORY
IN NAIVE AND MK-801 TREATED RATS

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Introduction

Cognitive function is markedly impaired in most patients with schizophrenia [1]. Working- long term memory impairment and attention deficits are the major symptoms of schizophrenia [2]. Typical antipsychotics have generally been reported to exert limited efficacy against cognitive impairments whereas atypical antipsychotics produce some degree of amelioration of cognitive dysfunction in schizophrenia [1,3]. N-methyl-D-aspartate (NMDA) receptor hypofunction is postulated to cause cognitive impairments, thus NMDA receptor antagonists, such as ketamine and phencyclidine, induce schizophrenia-like symptoms in healthy subjects, including positive, negative, and cognitive symptoms [3]. NMDA receptor antagonists also disturb learning and memory functions in animals that are similar to those seen in schizophrenia; these agents are useful for establishing animal models of cognitive impairment [4]. Controversily, a recent study found that, excessive glutamate release in the medial prefrontal cortex is associated with attentional deficits and confirmed that the suppression of glutamate release may be a target for the development of novel antipsychotic drugs with greater effect on some aspects of cognitive deficits [5].

Clinical studies showing the effects of risperidone and other antipsychotics on cognitive impairment in schizophrenic patients is also performed. To examine the influencing variables of neurocognition in patients with schizophrenia and to predict cognition during antipsychotic treatment 129 patients were treated with aripiprazole, olanzapine, quetiapine and risperidone. Quetiapine seemed to achieve the most favourable cognitive improvement [6]. Another study reported that, switching from oral atypical antipsychotics to long-acting injectable risperidone improved cognitive function in patients with schizophrenia [7].

Risperidone (RIS) is a second generation antipsychotic currently used in the treatment of acute psychosis and in the maintenance treatment of schizophrenia [8]. RIS, has a relatively wide-ranging profile of receptor interactions including dopaminergic and serotonergic
pathways. The main pharmacological activities of RIS include serotonergic 5-HT2A receptor blockade and dopaminergic D2 antagonism. Moreover it antagonizes histamine H1 receptors and α-1 adrenoreceptors to a lower extent [9]. RIS is less potent than the typical antipsychotic haloperidol as a central D2 antagonist in rats. Interaction with dopamine D1 receptors occurs only at very high concentrations. Although it interacts with histamine H1 and α-adrenergic receptors, it does not interact significantly with cholinergic receptors [10].

The present study was conducted in order to determine the effects of RIS on learning and memory in naive and MK-801 treated rats in different tasks: modified elevated plus maze (mEPM), passive avoidance (PA) and Morris Water Maze (MWM).

Materials and Methods

Animals

Adult male Wistar rats (Istanbul University Medical Sciences Research Center, DETAM, Turkey) weighing 200–250 g were housed five to six per home cages (37Lx28Wx18H) in an animal colony facility for 2 weeks before the start of the experiment. The animals were maintained in constant room temperature (22±2 °C) under a 12-h light/dark cycle (light onset at 07:00 h). Tap water and food pellets were provided ad libitum. All animals used for the experiments were naive to the tests used in this study. 136 rat was used in this study and each was tested individually and only once. Different groups of rats was used in each test and the number of animals per group ranged from 6-8.

All procedures for the treatment of animals were in compliance with the European Community Council Directive of 24 November 1986 and ethical approval was granted by the Ethics Committee of Kocaeli University (Number: KOU/HADYEK-10-7 Kocaeli, Turkey).
Drugs and treatments

RIS and MK-801 were purchased from Sigma Chemical Company (Sigma, St. Louis, MO). MK-801 was dissolved in 0.9% physiological saline and RIS was dissolved in saline using a few drops of 1% acetic acid [11]. Drugs were freshly prepared and administered by the intraperitoneal (i.p) route in a volume of 0.2 ml per 100 g body weight of rats.

The animals received saline (0.9%)+saline(0.9%), RIS (0.125 mg/kg)+saline(0.9%), saline(0.9%)+MK-801 (0.15 mg/kg) or MK-801 (0.15 mg/kg)+RIS (0.125 mg/kg) i.p. 60 and 30 min. before the first day's trial respectively (n=7-8 for each group) in mEPM test.

RIS (0.125, 0.25 mg/kg)+saline (0.9%), saline (0.9%)+MK-801 (0.15 mg/kg) and RIS (0.125 mg/kg)+MK-801 (0.15 mg/kg) was administered 60 and 30 min. respectively before the retention test of PA task (n=6-8 for each group).

In MWM test, twenty-four hours after the last acquisition session RIS (0.06, 0.125, 0.25 mg/kg) saline (0.9%), saline (0.9%)+MK-801 (0.15 mg/kg) or RIS (0.125 mg/kg) + MK-801 was administered 60 and 30 min. before the probe test respectively (n=6-7 for each group).

The animals received risperidone, MK-801 or MK-801 + risperidone (0.125 mg/kg) 60 and 30 min. before the locomotor activity test respectively.

The doses of drugs used in this study were selected on the basis of literature data which were previously reported [12].

Modified elevated plus-maze test

Cognitive behaviour was evaluated by using the mEPM learning task, which measures spatial long-term memory [13]. Transfer latency (the time in which the animal moves from the open arm to the enclosed arm) was used as an index of learning and memory processes.

The plus-maze was made of wood and consisted of two open arms (50×10 cm) surrounded by a short (1 cm) plexiglas edge to avoid falls, and two enclosed arms (50×10×40 cm) arranged such that two open arms were opposite to each other. The arms were connected by a central
platform (10×10 cm). The maze was elevated to a height of 50 cm above the floor. The open arms and central platform were painted white and enclosed arms were painted black. To remove any confounding olfactory cues the maze was cleaned with alcohol–water solution after each rat. The principle in this experiment is based upon the aversive behaviour of rodents to open and high spaces. The animals dislike open and high spaces and move from them to an enclosed arm, the protected areas of the maze.

The procedure was performed as described by the study of [14, 15, 16]. The animals were randomly assigned to the different experimental and control groups. In the acquisition session (on day 1), each rat was gently placed at the distal end of an open arm of the apparatus facing away from the central platform and the time it took for the rat to move from the open arm to either of the enclosed arms (transfer latency) was recorded. Training (repeated exposure of animal to open arms) shortens this parameter, possibly as a consequence of learning acquisition and retention. If the rat did not enter the enclosed arm within 90 s, it was excluded from further experimentation. The criterion of an animal’s entry into the enclosed arm was crossing with all four legs of an imaginary line separating the enclosed arm from the central space. After entering the enclosed arm, the rat was allowed to move freely in the maze regardless of open and enclosed arms for 10 s. Then, the rat was returned to its home cage. The retention session followed 24 h after the acquisition session (on day 2). The rats were put into the open arm and the transfer latency was recorded again. The experiments were conducted between 10:00 and 14:00 h in a semi-soundproof room.

**Passive-avoidance test**

Animals were trained in a one-trial, step-through, PA apparatus (Ugo Basile model 7551, Italy) for evaluating memory based on contextual fear conditioning and instrumental learning [17]. In this task the animal learns that a specific place should be avoided since it is associated
with an aversive event. Decrease in step through latency (STL) (retention latency) indicates an impairment in memory in the PA task.

The training apparatus consisted of two compartments, each measuring 22×21×22 cm. The illuminated white chamber was connected to the dark chamber (i.e. conditioning chamber) which was equipped with an electric cable grid floor and the shock was delivered to the animal's feet via a shock generator. The two chambers were separated by a flatbox partition, including an automatically operated sliding door at floor level.

Training trial was carried out as described by Hiramitsu et al and Monleon et al. [18, 19]. The animals received drugs prior to PA training. On the first day of training, rats were placed individually into the light compartment and allowed to explore the boxes. After 30 s, the door between these two boxes was opened and the animal moved into the dark compartment freely (preacquisition trial).

The acquisition (training) trial was carried out 15 min after the preacquisition trial. Rats were again placed in the light compartment of the PA apparatus. After a 10 s adaptation period in the safe chamber, the door between the compartments was opened. Having completely entered the dark compartment, the sliding door was closed automatically and an electric foot-shock (0.5 mA) of a 3 s duration was delivered to the animal via grid floor immediately. The time taken to enter the dark compartment was recorded (training latency). Any animal failing to cross from the illuminated to the dark compartment within 300 s was discarded from the experiment. Animals were then removed from the dark chamber and returned to their home cages. Between each training session, both compartments of the chamber were cleaned to remove any confounding olfactory cues.

Retention trial: Recall of this inhibitory stimulus was evaluated 24 h post-training by returning the animals into the light compartment and recording their latency to enter the dark compartment (four paws in). No foot-shock was applied in this trial. If the animal had not
entered to the dark compartment within 300 s, it was returned to its cage and a maximum latency of 300 s was recorded. This latency served as a measure of retention performance of the step-through avoidance response.

*Morris Water Maze test*

The MWM is a circular pool (150 cm diameter) filled with water (25°C) and small black plastic pieces were placed on the surface of the water in order to make the platform invisible. The pool was located in a dimly lit, soundproof test room with a number of extra-maze visual cues, including a white-black colored poster on the wall, a halogen lamp, a camera and the experimenter. The maze was divided into four quadrants [H, D, G and B (escape platforms quadrant)] and three equally spaced points were used as starting positions around the edge of the pool. The order of the release positions varied systematically throughout the experiment. An escape platform was located in one quadrant 1 cm above the water surface during the familiarization session and 1 cm below the water surface during the other sessions. The place of the platform was constant during the MWM test.

Videotracking conducted with a videocamera focused on the full diameter of the pool. Navigation parameters were analyzed by using the Ethovision 3.1 video analysis system (Noldus). The rats were trained in the MWM during one session per day on 5 consecutive days (S1, S2, S3, S4, S5).

One familiarization (the platform is visible) and four acquisition sessions (the platform is hidden) were performed in the MWM test. During the familiarization session and acquisition phase of the experiment, each rat was given three trials. The delay between the trials was 60 seconds and a 1 day interval was used between the sessions. For each daily trial, the rat was taken from the home cage and placed into the water maze at one of three randomly determined locations with its head facing the center of the water maze. After the rat had found and climbed on to the platform, the trial was stopped and the escape latency was recorded. If
the rat had not climbed onto the platform in 60 seconds, the trial ended automatically and the experimenter guided the rat to the platform and an escape latency of 60 seconds was recorded. A ‘probe trial’ was used to assess the animals spatial retention of the location of the escape platform.

The analyzed navigation parameters in the probe trial were time spent in escape platforms quadrant, distance to platform and swim speed. Time spent in escape platforms quadrant and distance to platform calculations were used as measures for the development of spatial memory, whereas the swim speed (cm/sec) parameter was used to assess locomotor function.

Locomotor activity test

Since compounds altering motor activity may give false positive / negative effects in these tests, an additional test was carried out with the specific aim of monitoring motor activity. The spontaneous locomotor activity of the animals was assessed by monitoring their activity in a locomotor activity cage (May 9803 Activity Monitoring System, Commat Iletisim Ltd. May Pentium Computer, Ankara, Turkey). Locomotion of the animals were measured immediately after the second day of elevated plus maze test for 5 min in the activity cage. Rats were individually placed in a plexiglas cage (42×42×30 cm) and the total number of movements were evaluated.

Statistical analysis

To compare the differences between transfer latencies of first and second day in a group in the mEPM test, Wilcoxon t-test was used. To elucidate the differences among drug treated groups during the first as well as during the second day transfer latencies in the m EPM test, Kruskal-Wallis non- parametric one-way analysis of variance (ANOVA) followed by Dunn’s test was used.
The latency for PA test was evaluated by two-way analyses of variance (ANOVA) \((\text{time} \times \text{treatment})\) followed by Dunnetts t-test. Scores of locomotor activity were also evaluated by two-way analyses of variance (ANOVA) followed by Dunnetts t-test.

The time spent in target quadrant, distance to platform and swim speed scores in MWM test were evaluated by two-way ANOVA, post-hoc Dunnetts t-test.

Results are expressed as mean±SEM. The criterian for statistical significance was \(p<0.05\).

**Results**

1. **Effect of RIS on the transfer latency of naive rats in the m EPM test**

Mean transfer latencies of RIS (0.125,0.25 mg/kg, i.p.) or vehicle was administered i.p. 60 min. before the first days trial in the m EPM test in rats are presented in Fig 1.

Second day transfer latencies significantly decreased compared to first day transfer latencies within control and RIS groups indicating that the rats remembered the presence of the enclosed arms in mEMP test. (#\(p<0.01\); Wilcoxon t-test, first day vs. second day). RIS had no effect on the transfer latencies on the first day (TL1) compared to that of the vehicle treated group (Kruskal–Wallis analysis \(H=0.89, p=0.64\)). On the second day RIS (0.125, 0.25 mg/kg) significantly decreased the transfer latency (TL2) of the animals *\(p<0.05\) (Kruskal–Wallis analysis: \(H=9.65, p=0.008\) followed by Dunn's test, vs. control group on the second day).

2. **Effects of RIS on the transfer latency of MK-801 treated rats in the m EPM test**

The effects of MK-801(0.15 mg/kg) given i.p. 30 min. before acquisition session on the transfer latencies of the first and second days are shown in Fig 2. There was no difference between first day (TL1) and second day (TL2) latencies compared within MK-801 treated group (Wilcoxon \(t\)-test, \(p=0.27\)).

MK-801 failed to change the transfer latency on the first day (Kruskal–Wallis analysis \(H=6.662, p=0.835\)), but significantly prolonged the transfer latency on the second day (TL2)
compared to that of the vehicle treated group (Kruskal–Wallis analysis H=18.74, * p<0.0002) indicating an impairment of learning and memory of the mEPM test in rats.

Although RIS (0.125 mg/kg) had no effect on the transfer latencies of MK-801 treated rats on the first day (Kruskal-Wallis analysis H=6.662, p = 0.835), it significantly reversed the MK-801 induced prolongation in the transfer latency of rats on the second day (TL2). (& p< 0.0032, vs MK-801 treated group, posthoc Dunn’s test).

3. **Effects of RIS on passive avoidance performance of naive rats or MK-801 treated rats**

During the training session (on day 1) of step-through type PA task, vehicle, RIS (0.125,0.25 mg/kg) or MK-801 (0.15 mg/kg) treated rats showed a similar STL (data not shown).

RIS slightly decreased STL but this parameter did not reach statistical significance (p=0.05) as compared to that of vehicle treated rats during the retention test, performed 24 h after the training test while MK-801 (0.15 mg/kg) significantly shortened STL on the test phase compared to control group in PA test (*p=0.02, Two way ANOVA, post-hoc Dunnetts t test F(4,30)=2.94. Decreased in STL indicates an impairment in memory retention of the PA task.

RIS failed to prolonged MK-801 induced reduction of retention latency in PA test (Fig 3).

4. **Effects of RIS on Morris water maze performance of naive rats and MK-801 treated rats**

In probe trial of MWM test, acute administration of RIS (0.06, 0.125, 0.25 mg/kg) had no effect on the time spent in target platform; MK-801 produced a significant decrease in this parameter ([F(5,32)=4.71, p=0.0023). RIS (0.125 mg/kg) tended to increase MK-801-induced reduction of the time spent in target platform but this finding did not reach statistical significance (Fig 4a).

While RIS (0.06, 0.25, 0.125 mg/kg) had no effect on distance to platform in MWM, MK-801 (0.15 mg/kg) prolonged this parameter (p<0.001). RIS (0.125 mg/kg) produced a significant reduction in distance to platform compared to MK-801 treated group F(5,32)=6.26, p=0.004). (Fig 4b).
When the effects of drugs on the swimming speed of the animals in the probe trial was compared there was no significant difference between the groups (F(5,32)=1.31, p>0.05) (Fig 4c). RIS (0.06, 0.25, 0.125 mg/kg) had no effect on the swimming speed. The effect of RIS on the motor function expressed as swimming speed also did not differ from the control group in MK-801 treated rats (Fig 4c).

5. Effects of RIS and MK-801 on locomotor activity of rats

Since the action of drugs on locomotor activity affects the outcome of the mEPM and PA tests, drug induced effects on locomotor activity of animals was determined. Locomotor activity of the animals was assessed by measuring the total number of movements for a 5 minute period. Statistical analyses of the present study showed that RIS (0.125, 0.25 mg/kg) and MK-801 (0.15 mg/kg) had no significant effect on the locomotor activity of rats. (Fig 5).

Discussion

In this study, we aimed to evaluate the effects of acute RIS treatment on three different behavioral tests of learning and memory and investigated the effects of RIS on the memory impairing effects of MK-801. The main finding of this study is that RIS augmented learning performance in untreated and MK-801 treated rats on the mEPM and reduced distance to platform in MK-801-treated rats in the MWM test.

In EPM test, the diminishment of the transfer latency on second days is used as a parameter for retention or consolidation of memory and treatment of drugs prior to first day is utilized for acquisition linked actions of drugs [20]. In this study, RIS (0.125, 0.25 mg/kg) affected memory positively and improved MK-801-induced memory deterioration in the retention session of mEPM test.

PA test is based on instrumental learning and contextual fear conditioning [17] which is dependent on hippocampus and amygdala [21]. It is commonly used for examining long-term emotional memory. In our study, RIS slightly decreased STL but this finding did not reach
statistical significance during the retention session which is performed 24 h after the training while MK-801 significantly decreased STL on the training session. Decreased STL in retention session indicates an impairment in memory. Moreover RIS had no effect on MK-801 induced deterioration of avoidance learning.

In MWM test, rats have to find complex behavioral strategies for swimming away from the pool wall, recognizing the platform as the only rescue from water, using the platform by climbing onto it, and remaining on the platform [22]. When the place of the escape platform is constant during the MWM test, the test evaluates hippocampal dependent reference memory [23] as in our study. Our results indicate that although RIS (0.06, 0.125, 0.25 mg/kg i.p.) had no effect on learning and memory in naive rats, it (0.125 mg/kg) reversed MK-801-induced learning impairment in MWM test.

Studies with antipsychotics reported various findings. Clozapine (CLZ) was reported to reverse scopolamine-induced cognitive dysfunction in PA and EPM paradigm [24] and MK-801 induced working memory impairment in radial arm maze test [25]. Olanzapine (OLZ) did not effect acquisition, consolidation or retrieval processes in PA test and improved spatial learning in MWM test. CLZ and haloperidol impaired acquisition and consolidation processes in PA test and spatial learning in MWM task in mice [26]. Lurasidone, a novel atypical antipsychotic drug was reported to reverse MK-801-induced impairment of learning and memory in PA, radial arm maze and MWM test [27]. Sertindole, in contrast to CLZ and OLZ, did not disrupt MWM performance after acute or chronic treatment [28]. Ziprasidone, OLZ, RIS and haloperidol markedly impaired spatial memory in MWM task [29]. The discrepancy between these results may be attributed to the methods used and the different strains of animals. Previous clinical studies indicate that RIS may have a positive effect on cognition [30]. Improvement in cognitive tests was found in schizophrenic patients treated with RIS [31].
RIS is an antipsychotic with balanced serotonin-dopamine antagonism [32,33]. It blocks 5-HT2A receptors with greater affinity than CLZ and haloperidol does and it also antagonizes histamine H1 receptors, α-1 and α-2 adrenoceptors to a lower extent [34].

Serotonergic system appears to play a key role in behaviors that involve a high cognitive demand and in memory improvement or recovery from impaired cognitive performance [35]. In the reversal effect of RIS on MK-801-induced learning deficits several mechanisms may play role. 5-HT$_2$A receptors are DA inhibitors and by this way 5-HT$_2$A antagonism stimulates dopamine release in certain brain areas. Effects of serotonin on dopaminergic pathways might play an important role in relieving cognitive deficits [36, 37].

NMDA receptor hypofunction of schizophrenia is a powerful hypothesis that underlies progressive loss of neuronal function. Antipsychotics are postulated to effect glutamatergic neurotransmission by modulating the release of glutamate by altering the density or subunit composition of glutamate receptors [38]. Blockade of 5HT2A receptors would reduce glutamate release which in turn would reduce mesolimbic dopamine release. Our results support the hypothesis that 5HT2A interaction with NMDA receptors is therefore a possible mechanism for reducing cognitive deficits.

RIS irreversibly binds to and exhibits a high affinity for the 5-HT7 receptor and it is reported that 5-HT7 receptor might provide a target for the treatment of psychotic disorders [39]. Therefore, the therapeutic activity of RIS in cognition in MK-801 administered rats may be linked to the 5-HT7 receptor blockade of the drug.

In conclusion our results indicated that RIS augmented learning performance in untreated and MK-801-treated rats on the modified elevated plus maze and reduced the distance to the platform in MK-801-treated rats in the Morris water maze test. Thus, RIS seems to play an important role in improving cognitive dysfunctions related to NMDA receptors.
References


Figure Legends:

Fig 1: Effect of risperidone on the transfer latencies (of first day and second day) to the enclosed arm of the elevated plus-maze in rats. The number of rat is shown in the columns. Transfer latency data (s) are expressed as mean±SEM values.

Fig 2: Effects of risperidone and MK-801 on the transfer latencies (of first day and second day) to the enclosed arm of the elevated plus-maze in rats and reversal of the MK-801-induced impairment in the transfer latencies of rats by risperidone. The number of rats is shown in the columns (n=7-8). Transfer latency data (s) are expressed as mean±SEM values.

Fig 3: Effects of risperidone, MK-801 and concurrent administration of risperidone and MK-801 on the STL of rats during retention test of passive-avoidance task. The number of rat is shown in the columns (n=6-8). Retention latency data (s) are expressed as mean±SEM values.

Fig 4: Effects of risperidone, MK-801 and concurrent administration of risperidone and MK-801 on MWM performance. The number of rat is shown in the columns. The data are expressed as mean±SEM values.

(a) The time spent in target quadrant in the probe trial of MWM test
(b) Distance to platform in the probe trial of MWM test
(c) Swimming speed in the probe trial of MWM test.

Fig 5: Effects of risperidone and MK-801 on locomotor activity of animals. The number of rats is shown in the columns. Scores are expressed as mean±SEM values.