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Research report

GLYX-13 (rapastinel) ameliorates subchronic phencyclidine- and ketamine-induced declarative memory deficits in mice

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HIGHLIGHTS

- GLYX-13 (rapastinel), a putative fast-acting anti-depressant, was effective in reversing subchronic phencyclidine (PCP)- and ketamine-induced persistent deficits in novel object recognition (NOR) task in mice.
- Acute ketamine, but not rapastinel, caused a transient NOR deficit, which was prevented by pretreatment with rapastinel.
- Rapastinel potentiated the ability of a sub-effective dose of lurasidone, an atypical antipsychotic drug with antidepressant properties, to restore NOR in subchronic ketamine-treated mice.
- The differences in rapastinel and ketamine on this type of cognitive function in mice are noteworthy and may be relevant to safety issues in man.

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ABSTRACT

GLYX-13 (rapastinel), a tetrapeptide (Thr-Pro-Pro-Thr-amide), has been reported to have fast acting antidepressant properties in man based upon its *N*-methyl-D-aspartate receptor (NMDAR) glycine site functional partial agonism. Ketamine, a non-competitive NMDAR antagonist, also reported to have fast acting antidepressant properties, produces cognitive impairment in rodents and man, whereas rapastinel has been reported to have cognitive enhancing properties in rodents, without impairing cognition in man, albeit clinical testing has been limited. The goal of this study was to compare the cognitive impairing effects of rapastinel and ketamine in novel object recognition (NOR), a measure of declarative memory, in male C57BL/6J mice treated with phencyclidine (PCP), another NMDAR noncompetitive antagonist known to severely impair cognition, in both rodents and man. C57BL/6J mice given a single dose or subchronic ketamine (30 mg/kg. i.p.) showed acute or persistent deficits in NOR, respectively. Acute i.v. rapastinel (1.0 mg/kg), did not induce NOR deficit. Pre-treatment with rapastinel significantly prevented acute ketamine-induced NOR deficit. Rapastinel (1.0 mg/kg, but not 0.3 mg/kg, iv) significantly reversed both subchronic ketamine- and subchronic PCP-induced NOR deficits. Rapastinel also potentiated the atypical antipsychotic drug with antidepressant properties, lurasidone, to restore NOR in subchronic ketamine-treated mice. These findings indicate that rapastinel, unlike ketamine, does not induce a declarative memory deficit in mice, and can prevent or reverse the ketamine-induced NOR deficit. Further study is required to determine if these differences translate during clinical use of ketamine and rapastinel as fast acting antidepressant drugs and if rapastinel could have non-ionotropic effects as an add-on therapy with antipsychotic/antidepressant medications.

Abbreviations: 5-HT, serotonin; AAPD, atypical antipsychotic drugs; CHPG, (*RS*)-2-chloro-5-hydroxyphenylglycine; CI, cognitive impairing; CIS, cognitive impairment associated in schizophrenia; DA, opamine; DI, discrimination index; GABA, gamma amino butyric acid; Ket, ketamine; LTD, long-term depression; LTP, long-term potentiation; NMDAR, *N*-methyl-D-aspartate receptor; NOR, novel object recognition; GluN2A, NMDR 2-subunit A; GluN2B, NMDR 2-subunit B; PCP, phencyclidine; Sal, saline; WT, wild-type.

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1. Introduction

Rapastinel, an NMDA receptor modulator with glycine-site partial agonist properties [1], has been reported to have rapidly acting antidepressant properties [2]. Ketamine, an NMDAR non-competitive antagonist, has also been reported to produce rapidly acting antidepressant properties [3]; however, it also causes dissociative and psychotic-like effects, as well as cognitive impairment, in healthy humans [4,5], and exacerbates psychosis, but not cognitive impairment, in patients with schizophrenia [6–8]. Ketamine also causes deficits in cognition in rodents, including novel object recognition (NOR), an analog of human declarative memory [9]. NOR is dependent on the integrated action of the hippocampus, entorhinal, perirhinal and temporal association cortices, and prefrontal cortex [10].

By contrast with ketamine, rapastinel has been reported to enhance hippocampal-dependent spatial learning tasks in rodents [11], indicating it may have cognitive enhancing, as well as antidepressant properties [1]. However, there is no data on the effects of rapastinel on neuropsychological test performance in normal volunteers or patients with depression.

Glutamate, via its actions at NMDAR and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptors, has a profound effect on synaptic plasticity and, thus, plays a major role in learning and memory [12]. Subchronic treatment with the NMDAR antagonist, phencyclidine (PCP), induces significant deficits in NOR in both rats and mice [13–16]. This deficit is reversed by atypical antipsychotic drugs (AAPDs), including lurasidone [16], which is also an effective antidepressant in patients with bipolar depression [17] and has a small, but statistically significant effect, on depression ratings in patient with schizophrenia as well [18].

The goal of this study was to assess the effects of rapastinel on NOR in C57BL/6J mice and to determine if it could block or reverse the cognitive impairing effect of ketamine and PCP on NOR.

We chose ketamine rather than PCP for some experiments since ketamine and rapastinel are both being developed as antidepressants but PCP is not. We chose PCP for some studies to show that ketamine is like PCP and both impair cognition whereas rapastinel does not. We also sought to determine the interaction of rapastinel with the AAPD/antidepressant, lurasidone, due to its potential as an add-on therapy.

2. Materials and methods

2.1. Animals

Three cohorts of male C57BL/6J mice (2–1/2–3 month old, Jackson, MA, USA), $N = 40$, 45, and 48 were used in experiments 1–3 respectively. Mice were group housed (five/cage) in a controlled environment held at $21 \pm 2\,^{\circ}C$ and $50 \pm 15\%$ relative humidity with a 14:10 h light–dark period (lights on: 05:00 am). All experiments were conducted during the light phase. Food and water were available ad libitum . The mice were habituated to the colony upon arrival for a week, during which time, they were not handled. All experiments were conducted in accordance with Institutional Animal Care and Use Committee of Northwestern University, Chicago.

2.2. Drugs

Rapastinel was obtained from SAI Life Sciences (India). PCP was a generous gift from National Institute of Drug Abuse. Ketamine was purchased from Sigma–Aldrich (St. Louis, MO). Lurasidone was provided by Sumitomo Dainippon Pharma Co., Ltd. (Osaka, Japan). Rapastinel, PCP, and ketamine were dissolved in 0.9% sterile saline (Sal). PCP and ketamine were administered intraperitoneally (ip), at a volume of 10 mL/kg body weight. Rapastinel was given intravenously (iv). The dose of rapastinel (1.0 mg/kg) was chosen because it produced optimal enhancement in learning in both young adult and aged rats [11] and in a trace eye blink conditioning task in rabbits [19]. The doses for ketamine (30 mg/kg) and PCP (10 mg/kg) were chosen based on prior studies, which showed that these doses induce significant cognitive impairment in mice and rats [20,21]. The dose for lurasidone (0.1 mg/kg) was chosen based on prior NOR studies in C57BL/6J mice which determined the effective dose of lurasidone to restore NOR in subchronic PCP-treated mice [16].

2.3. Drug treatment

For acute drug treatments, rapastinel (1.0 mg/kg, iv), lurasidone (0.1 mg/kg, i.p.) or ketamine (30 mg/kg, ip) were administered 30 min prior to the acquisition trial of the NOR task (described below) to the subchronic ketamine or subchronic PCP-treated animals. For subchronic drug treatments, 7–10 mice/cohort were randomly assigned to Sal, PCP, or ketamine. The Sal-treated mice received 0.9% NaCl; the drug treatment groups received either PCP (10 mg/kg; ip), or ketamine (30 mg/kg; ip) twice daily for 7 days. This was followed by a 7 day washout period during which time, mice were left undisturbed in the home cage until initiation of habituation (see below).

2.4. NOR test

NOR testing in mice was slightly modified from Hashimoto et al. [20] (*i.e.*, size of the box, usage of white background to the walls of the box instead of black reflective surfaces, and duration of the trials) based on preliminary experiments. Our studies showed that when black reflective surfaces were used for the inner surfaces of the NOR box, the animals failed to explore much. Similar observations were made when large objects were used. Hence, we used white walls for the box and small objects for exploration. The dimensions of the NOR box we used for mice is comparable to that of rats. We used box of small dimensions as mentioned by Hashimoto et al. and did not find any significant difference in the object exploration when the dimensions of the NOR box was bigger [20]. We also observed in our preliminary studies, that C57BL/6J mice explored less when the duration of trials were 3 or 5. Hence, we let the animals explore for 10 min in both trials and noticed significant increase in exploration times (unpublished data). Hence we used longer duration of the trials. The NOR apparatus consisted of an open box made of Plexiglas (52 cm L; 52 cm W; 31 cm H) with white walls and a solid floor. The box was positioned approximately 30 cm above the floor, centered on a table such that the overhead lights could not provide a spatial cue. One day after the 7 day washout from subchronic drug treatment or Sal, mice were habituated to the empty NOR arena, as a group, for one hour, on each of three days prior to the acquisition trial. During the acquisition trial, the mice were allowed to explore two identical objects (*e.g.*, A1 and A2) for 10 min. This was followed by a 24 h inter-trial interval, after which the mice were returned to the home cage. During the retention trial, the mice were allowed to explore the familiar object (A) from the acquisition trial and a novel object (*e.g.*, B). The location of the novel object in the retention trial was randomly assigned for each mouse tested using a pseudorandom schedule. The pseudorandom sequences followed the criteria suggested by Gellerman to reduce the effects of object and place preference [22]. Also, to avoid bias or olfactory trails, we used an objects in triplicates, *i.e.*, that the same object that was used in the acquisition trial was not presented in retention trial. Behavior was recorded on video for blind scoring of object exploration. Object exploration was defined as an animal licking, sniffing, or touching the object with the forepaws while