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Research report

Combination of behaviorally sub-effective doses of glutamate NMDA and dopamine D_1 receptor antagonists impairs executive function

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HIGHLIGHTS

- NMDA and D_1 receptor blockade act synergistically to cause behavioral inflexibility and perseveration.
- Subtle abnormalities of glutamatergic and dopaminergic systems are sufficient to cause executive functional deficits.
- Executive function is more sensitive to combined NMDA and D_1 receptor dysfunction than learning or memory retrieval.

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ABSTRACT

Impairment of executive function is a core feature of schizophrenia. Preclinical studies indicate that injections of either *N*-methyl D-aspartate (NMDA) or dopamine D_1 receptor blockers impair executive function. Despite the prevailing notion based on postmortem findings in schizophrenia that cortical areas have marked suppression of glutamate and dopamine, recent *in vivo* imaging studies suggest that abnormalities of these neurotransmitters in living patients may be quite subtle. Thus, we hypothesized that modest impairments in both glutamate and dopamine function can act synergistically to cause executive dysfunction. In the present study, we investigated the effect of combined administration of "behaviorally sub-effective" doses of NMDA and dopamine D_1 receptor antagonists on executive function. An operant conditioning-based set-shifting task was used to assess behavioral flexibility in rats that were systemically injected with NMDA and dopamine D_1 receptor antagonists individually or in combination prior to task performance. Separate injections of the NMDA receptor antagonist, MK-801, and the dopamine D_1 receptor antagonist, SCH 23390, at low doses did not impair set-shifting; however, the combined administration of these same behaviorally sub-effective doses of the antagonists significantly impaired the performance during set-shifting without affecting learning, retrieval of the memory of the initial rule, latency of responses or the number of omissions. The combined treatment also produced an increased number of perseverative errors. Our results indicate that NMDA and D_1 receptor blockade act synergistically to cause behavioral inflexibility, and as such, subtle abnormalities in glutamatergic and dopaminergic systems may act cooperatively to cause deficits in executive function.

1. Introduction

Executive function is a complex phenomenon comprising attention, working memory, planning, reasoning, sequencing, inhibitory control and cognitive flexibility [1]. Severe impairment of executive function is a core feature of schizophrenia, and is an important determinant of long-term outcome and quality of life of these patients [2–5]. In addition to challenges with daily activities, patients with impaired executive function demonstrate difficulty performing standardized neuropsychological assessments of behavioral flexibility, such as the Wisconsin Card Sorting Test (WCST) [6,7]. Based on the findings from functional neuroimaging studies, a variety of cortical and subcortical areas have been implicated in the successful performance of the WCST, most notably the dorsolateral prefrontal cortex (DLPFC) anterior cingulate cortex, striatum, hippocampus and the mediodorsal nucleus of the thalamus [8–11]. Similarly, preclinical studies in rodents have confirmed that the aforementioned brain regions are necessary for behavioral flexibility during such tasks as set-shifting [12–17].

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Postmortem studies on schizophrenic brains have demonstrated a significant loss of glutamic acid decarboxylase-67 (GAD67) expression across multiple cortical areas indicating decreased activity of γ-amino butyric acid (GABA) [18–21]. Furthermore, a marked loss of dopaminergic fibers was described in the DLPFC of postmortem brains of schizophrenia [22], and there was a severe loss of dendritic spines within the prefrontal cortex and the hippocampus, indicating possible loss of glutamatergic synapses in schizophrenia [23]. Decreased levels of a number of proteins associated with glutamatergic synapses in the thalamus were also identified [24]. In addition, a significant loss of neurons has been described in the mediodorsal nucleus of the thalamus in schizophrenia brains [25]. Overall, available postmortem studies of schizophrenia, despite the heterogeneity of the disorder, point to a severe loss of glutamatergic, dopaminergic and GABAergic function in multiple brain areas in schizophrenia. Consistent with the findings, preclinical studies using set-shifting tasks have revealed that the separate administration of relatively high doses of either D_1 dopamine, *N*-methyl D-aspartate (NMDA) or GABA-A receptor antagonists worsens performance [26–29], suggesting that decreased activity of any one of these neurotransmitters could underlie the impaired behavioral flexibility in schizophrenia.

Although the collective results of postmortem studies on schizophrenia brains and preclinical models suggest that severe deficits in GABA, dopamine or glutamate function may be responsible for the impairments in executive function observed in schizophrenia, the results of *in vivo* imaging studies on schizophrenic patients consistently provide a contradictory view. Studies using *in vivo* magnetic resonance spectroscopy (MRS) or positron emission tomography (PET) have identified that levels of glutamate, dopamine and GABA in the DLPFC and thalamus are not different or slightly elevated in schizophrenia patients in comparison to control groups [30–40]. Based on the above MRS and PET findings, it is conceivable that the extent of suppression of neurotransmitter function predicted based on postmortem findings may not apply in patients living with schizophrenia, and if at all, the differences may be subtle, perhaps due to compensatory mechanisms. Furthermore, recent theories postulate that certain GABA abnormalities in schizophrenia might be compensatory [41,42]. Consequently, we hypothesized that subtle abnormalities in the glutamate and dopamine neurotransmitter systems in functionally-connected cortical and subcortical areas may cooperate synergistically to impair executive function in schizophrenia; an experimental consideration which had not been addressed in previous preclinical rodent models as they only targeted a single neurotransmitter system at a given time.

Among dopamine receptors, D_1 receptor subtype is more commonly implicated in executive function [15,29]. In addition, *in vivo* imaging studies have identified increased D_1 receptor levels in the DLPFC in schizophrenia patients [43]. Consequently, in the present study, we focused on D_1 receptor antagonism. Ultimately, we investigated the potential synergistic effect of the combined administration of "behaviorally sub-effective" doses of D_1 and NMDA receptor antagonists on set-shifting in rats performing a lever-pressing task that required them to shift between visual-cue and egocentric spatial response-based discrimination strategies according to the paradigm of Enomoto et al. [28]. To that end, in separate groups of rats, we first determined the doses of the D_1 antagonist, SCH 23390, and NMDA uncompetitive antagonist, MK-801, which when systemically administered alone, failed to worsen set-shifting performance compared to vehicle-treated controls. Next, these behaviorally sub-effective doses were co-administered systemically to a separate group of rats, and the results compared to controls as well as rats that received a higher (i.e., "effective") dose of MK-801 delivered alone.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (Charles River, Quebec, Canada) weighing 325–350 g at the beginning of the study were housed individually in an animal facility with temperature and humidity controlled rooms ($24 \pm 2\,^{\circ}C$, relative humidity $55 \pm 10\%$), 12-h light/dark cycle (lights on at 7:00 a.m.). Animals were food restricted to ~85% of their free feeding body weight. During food restriction, rats were weighed and handled for several minutes per day to get familiarized to handling by the investigator. All procedures were approved by the Institutional Animal Care Committee and are in compliance with the Canadian and National Institute of Health Guides for the Care and Use of Laboratory Animals (NIH Publication #80-23).

2.2. Drugs

The glutamate NMDA receptor antagonist MK-801 [(+)-MK-801 maleate, MWt: 337; (dizocilpine); 0.075 mg/kg or 0.05 mg/kg of the salt form; Sigma-Aldrich, St. Louise, MO], and dopamine D_1 antagonist SCH 23390 [(R)-(+)-SCH 23390 hydrochloride, MWt: 324; 0.005 mg/kg of the salt form; Sigma-Aldrich] were used, with the doses chosen based on pilot studies. Despite a small difference in concentration (0.18 on a Log10 scale), 0.075 mg/kg of MK-801 consistently affected set-shifting compared to 0.05 mg/kg dose in pilot studies. Thus, in the present study, these doses of MK-801 were employed and referred to as "effective" and "behaviorally sub-effective" doses, respectively. Drugs were freshly prepared and dissolved in physiological saline. Rats received subcutaneous injections of drugs individually or in combination on the day of response discrimination (i.e., set-shifting), 25 min prior to the visual-cue retrieval trials. In pilot studies, higher doses of SCH 23390 resulted in gross motor deficits, which rendered the animals incapable of performing the visual-cue and set-shifting tasks, and therefore, experiments using these higher doses of SCH 23390 were not included in the present study.

2.3. Apparatus

The operant conditioning apparatus (Med-Associates, St. Albans, VT, USA) consisted of a modular acrylic test chamber ($30.5 \times 24 \times 21$ cm), housed in a sound-attenuating box. The test chamber was equipped with grid floor, two retractable levers on either side of a central pellet receptacle, and a house light (white, 100-mA, located centrally on the top of the wall opposite to the levers). Positioned above each lever was a cue light (light emitting diode). Following a lever-press that was considered a correct response, a pellet dispenser dropped a sucrose pellet (45 mg; BioServ, Frenchtown, NJ) into the central pellet receptacle. The operation of the chamber was controlled by a customized computer software program (MED-PC IV, Med-Associates).

2.4. Set-shifting

Behavioral flexibility was assessed in rats using an operant conditioning-based a set-shifting task developed by Floresco et al. [14], with minor modifications. As described in detail below, rats were exposed to a series of experimental steps which included acclimatization to the chamber, training to press the levers, determination of the rat's preference for one lever over the other (i.e., its side bias), visual-cue discrimination ability, and finally, response discrimination ability to assess behavioral flexibility (Fig. 1). Each rat completed this entire series of experimental steps only once.