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Ventral hippocampal $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptor blockade and clozapine effects on memory in female rats

Ana Pocivavsek, Laura Icenogle, and Edward D. Levin[□]

Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC 27710, USA e-mail: edlevin@duke.edu

Abstract

Rationale— Nicotinic systems in the hippocampus play important roles in memory function. Decreased hippocampal nicotinic receptor concentration is associated with cognitive impairment in schizophrenia and Alzheimer's disease.

Methods— We modeled in rats the cognitive effects of chronic decrease in hippocampal $\alpha 7$ or $\alpha 4\beta 2$ receptors with 4-week continuous bilateral local infusions of the $\alpha 7$ nicotinic antagonist methyllycaconitine (MLA) or the $\alpha 4\beta 2$ antagonist dihydro- β -erythroidine (DH β E). The working memory effects of these infusions were assessed by performance on the radial-arm maze. To test the effect of antipsychotic medication, we gave acute injections of clozapine and to determine the impact of nicotine, which is widely used by people with schizophrenia approximately half of the rats received chronic systemic infusions of nicotine.

Results— Chronic ventral hippocampal DH β E infusion caused a significant ($p < 0.001$) working memory impairment. Acute systemic clozapine (2.5 mg/kg) caused a significant ($p < 0.005$) working memory impairment in rats given control aCSF hippocampal infusions. Clozapine significantly ($p < 0.025$) attenuated the memory deficit caused by chronic hippocampal DH β E infusions. Chronic ventral hippocampal infusions with MLA did not significantly affect the working memory performance in the radial-arm maze, but it did significantly ($p < 0.05$) potentiate the memory impairment caused by 1.25 mg/kg of clozapine. Chronic systemic nicotine did not significantly interact with these effects.

Conclusions— The state of nicotinic receptor activation in the ventral hippocampus significantly affected the impact of clozapine on working memory with blockade of $\alpha 7$ nicotinic receptors potentiating clozapine-induced memory impairment and blockade of $\alpha 4\beta 2$ receptors reversing the clozapine effect from impairing to improving memory.

Keywords

Nicotine; Clozapine; Working memory; Radial-arm maze; MLA; DH β E; Hippocampus

Introduction

Nicotinic involvement in cognitive function has been widely investigated with a variety of animal and human studies showing that nicotine can improve learning and memory performance and nicotinic antagonists impair memory (Brioni et al. 1997; Buccafusco et al. 1996; Levin 1992; Levin et al. 1996b; Levin and Simon 1998; Newhouse et al. 1997). Memory improvement induced by nicotine appears to affect working memory with limited noticeable effect on reference memory (Levin et al. 1996a, 1997). The specific subtypes and anatomic

E. D. Levin, Department of Psychiatry, Neurobehavioral Research Laboratory, Duke University Medical Center, P.O. Box #3412 Durham, NC 27710, USA.

localization of nicotinic receptor involvement in memory is still being determined. The best understanding of nicotinic receptor subtype involvement in memory concern nicotinic receptors in the hippocampus. The $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors in the ventral hippocampus are critical for spatial working memory (Bancroft and Levin 2000; Bettany and Levin 2001; Felix and Levin 1997; Levin et al. 2002). Hippocampal nicotinic receptors appear to be critically involved in the cognitive impairments of schizophrenia and Alzheimer's disease.

Schizophrenia is characterized not only by psychosis, but also by pronounced cognitive impairment. This cognitive impairment is associated with dysfunction of hippocampal nicotinic $\alpha 7$ receptors with possible involvement of $\alpha 4\beta 2$ receptors as well (Addington 1998; Durany et al. 2000). Nicotinic systems may be critical for the development of effective therapy for cognitive impairment in schizophrenia. Significant deficits in nicotinic receptors have also been found in Alzheimer's disease (Kellar et al. 1987; London et al. 1989; Nordberg et al. 1988; Perry et al. 1987; Schroder et al. 1995; Whitehouse and Kalara 1995). Alzheimer's disease is principally considered a disorder of cognitive function. However, psychotic disturbances are frequently seen in Alzheimer's disease as well and antipsychotic drugs are used to treat these symptoms. The development of cognitive enhancing for schizophrenia and Alzheimer's disease must consider interactions with the antipsychotic treatments also in use.

We have determined that transdermal nicotine administration significantly improves the attention of patients with schizophrenia and also it offsets the memory impairment caused by the antipsychotic drug haloperidol (Levin et al. 1996c). Experimental rat studies have also found reversal of chronic haloperidol-induced memory impairments with nicotine administration (Levin 1997). Acute coadministration of haloperidol with nicotine decreased the nicotine-induced memory improvement (Addy and Levin 2002). In a similar fashion, the antipsychotic drug risperidone also attenuated nicotine-induced memory improvements. Like haloperidol, acute risperidone alone at the doses given did not impact the memory performance; however, coadministration of risperidone with nicotine significantly reversed the original nicotine-induced memory improvement (Addy and Levin 2002). Interactions between nicotine and haloperidol and risperidone, which have pronounced dopamine antagonist effects, likely involve nicotine-induced dopamine release (Wonnacott et al. 1989).

Atypical antipsychotic drugs like clozapine have a wide variety of receptor actions. It is difficult to theoretically posit the functional nature of clozapine–nicotinic interactions relevant for cognitive function. Experimental studies can help determine the neural bases for nicotinic–antipsychotic drug interactions. We have previously found that the clozapine-induced memory impairments in rats can be reversed by acute nicotine coadministration (Addy and Levin 2002).

In contrast to clozapine-induced memory and attentional impairment seen in intact experimental animals (Addy and Levin 2002; Rezvani et al. 2004), clozapine has been seen in some studies to improve cognitive function in patients with schizophrenia. Treatment of patients with schizophrenia with clozapine has been found to improve psychomotor speed, verbal fluency, verbal learning, and memory (McGurk 1999). Given that antipsychotic drugs such as clozapine are given therapeutically to patients with brain dysfunction, it is important to consider clozapine interactions with compromised brain function. A critical component in the nature of clozapine effects on cognitive function appears to be the functionality of the hippocampus. Clozapine doses, which cause significant memory impairment in intact rats, significantly attenuate the memory impairment caused in rats by lesions to the fimbria fornix a principal connection of the hippocampus with the septum, which provides cholinergic innervation to the hippocampus (Addy et al. 2005).

The aim of the current study was to analyze neural mechanisms that underlie the cognitive effects of nicotinic interactions with antipsychotic drugs, specifically examining changes in memory function. Clozapine has been widely used in schizophrenia where there is a nicotinic $\alpha 7$ deficit and Alzheimer's disease where there is a nicotinic $\alpha 4\beta 2$ deficit. It is important to know its differential effects on cognition under the conditions of both $\alpha 7$ and $\alpha 4\beta 2$ deficiency. We investigated the cognitive effects of clozapine under conditions of chronic nicotinic receptor deficiency such as what occurs in schizophrenia and Alzheimer's disease and chronic nicotine administration such as what occurs in schizophrenia. Further analysis of neural mechanisms involved in these nicotine interactions can aid the development of antipsychotic drugs with fewer cognitive side effects, as well as aid the improvement of cognitive function associated with schizophrenia as well as Alzheimer's disease.

Methods

Subjects

Young adult female Sprague-Dawley rats (Taconic Farms) between 200 and 300 g were used. Female rats were used to match our line of studies, in which we have determined the effects of antipsychotic drugs on cognitive function. In females, the potential interfering effect of sedation does not appear to be as much of a problem as with males. The rats were housed in groups of three during initial maze training and then one per cage after cannulation. The rat colony room had a reverse 12-h light/12-h dark cycle in which the lights were off during the daytime so that the nocturnal rats were behaviorally active when tested during the dark phase. The rats had ad libitum access to drinking water and were fed daily after testing which kept them at a healthy lean weight and motivated for the food reinforcements used in the radial-arm maze. The experiments conformed to Duke University and federal guidelines on the ethical use of animals.

General design

The importance of the ventral hippocampal nicotinic receptors for memory was examined with chronic local infusions of the nicotinic $\alpha 7$ antagonist methyllycaconitine (MLA) or the nicotinic $\alpha 4\beta 2$ antagonist dihydro- β -erythroidine (DH β E) given together with systemic chronic nicotine and acute clozapine actions. The drug effects on working memory were assessed using the 8-arm radial maze. Rats were trained for 18 sessions to asymptotic levels of choice accuracy as measured by the number of correct entries before an error (entries to repeat). Chronic bilateral infusion cannulae were implanted into the ventral hippocampus using stereotaxic techniques. At the same time, osmotic minipumps were implanted to chronically infuse nicotine. For a week after surgery, the animals were trained and tested for adverse effects resulting from the surgery. Clozapine was then injected acutely (sc) at doses of 0, 1.25, and 2.5 mg/kg each week (weeks 2–4 of systemic nicotine exposure and hippocampal nicotinic antagonist infusion) in a counterbalanced order, 20 min before testing to determine clozapine effects on the radial-arm maze choice accuracy. Performance scores were averaged across the 3 weeks of testing to provide more stable and complete measurements. After the drug sessions were completed, we killed the rats and verified cannulae placement using histological methods.

Radial-arm maze

Cognitive tests were performed using a black, wooden 8-arm radial maze. The maze was elevated 30 cm off the floor with a central platform 35 cm in diameter and 8 arms each 10×80 cm. Each arm contained a food cup, at its terminal end, which was baited during testing with 1/2 piece of sweetened cereal (Froot Loops®, Kellogg's, Battle Creek, MI, USA). Then a 30-cm opaque ring was placed on the central platform and a rat was placed inside the ring for 10 s. After this interval, the ring was removed and timing began. The rat was allowed to run on the maze until all 8 arms were entered or until 300 s had passed. An arm entry was recorded

when all four of the animal's legs had crossed the threshold of the arm. Choice accuracy was measured by entries to repeat (ETR), which was the number of arms entered until a repeat entry was made in a previously chosen arm. For the session to be included in the analysis, the rat had to either have a repeated entry, finish the maze perfectly with no repeated entries, or have chosen more than half of the arms before the end of the session. If there was no repetition and more than half of the arms were not entered, the session was not counted and the choice accuracy of that rat for the other sessions with the same clozapine dose were used to calculate the average performance. Response latency was recorded as the total time divided by the number of arm entries. The square root transformation was used to reduce the heterogeneity of variance in latency measurements. As consistently high levels of choice accuracy were observed after 18 sessions, the rats were cannulated. The rats were divided into matched treatment groups based on the choice accuracy. The rats were trained 4–5 days per week.

Cannulation

Twenty-four rats were cannulated in the ventral hippocampus. The rat was anesthetized with an i.p. injection of ketamine (75 mg/kg) and medetomidine (0.3 mg/kg) mixture. Before beginning surgery, the rat's head was shaved and it was placed on a heating pad to maintain body temperature constant. After induction of anesthesia, the rat was secured onto the stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) with ear bars and a bite bar the elevated the head at an angle 5 mm above the intraaural line. A longitudinal incision was made over the scalp. The bregma was used as the point of reference for the ventral hippocampus coordinates, obtained from the Pellegrino atlas (Pellegrino et al. 1979). The guide cannulae were lowered through holes drilled in the skull to the following coordinates from bregma: AP -3.2 , ML ± 5.0 , and DV -7.0 . The guide cannulae were aimed 1 mm dorsal to the intended site of infusion to minimized damage to the target structures. Four screws were anchored in the skull between the cannulae; wire was wrapped around the screws. These were used to help anchor the protective cap that was formed by cranioplastic cement. The cement, a two-part acrylic-based medium, was applied to surround and secure the cannulae; it was also used to cover the incision area. The rats were given 1 week of rest after surgery before being run on the maze. Two postsurgery trials were run to assess the rats' performance, establishing any surgery-related effects. To familiarize the rats with the infusion procedure, they were given two saline infusions.

Drug treatment

Chronic drug administration was used for hippocampal infusion of nicotinic antagonists and systemic administration of nicotine to investigate the clozapine effects on memory in a neural system adapted to chronic nicotine as is the case in schizophrenia most of whom smoke and central nicotinic receptor deficits such as is seen in schizophrenia and Alzheimer's disease. The hippocampus was chosen for investigation because of its importance for memory function. Osmotic minipumps (Alzet, 2004, Alza, Palo Alto, CA, USA), with a volume delivery rate of $0.25 \mu\text{l/h}$, were subcutaneously implanted in the rats at the same time as the cannulation procedure. One pump was connected to each of the infusion cannulae with PE tubing. Half of the rats were infused with artificial cerebral spinal fluid (aCSF) and the other half received DH β E at a rate of $100 \mu\text{g/side per day}$ or MLA at a rate of approximately $82 \mu\text{g/side per day}$ with a M2004 pump continuously for 4 weeks. Approximately half of each of those groups received systemic nicotine treatment with minipumps (Model 2ML4) with a delivery rate of $2.5 \mu\text{l/h}$ containing nicotine (Sigma, St Louis, MO, USA), dissolved in 0.9% sterile saline, which delivered the nicotine ditartrate (dose measured as of the base weight) at a rate of approximately $5 \text{ mg kg}^{-1} \text{ day}^{-1}$. Control rats received minipumps containing sterile saline only. For a 4-week period, the minipumps administered the chronic nicotine or vehicle infusion and the drug studies were also conducted during this period. The final group sizes for each

treatment group with each having correct bilateral local drug infusion cannula placements on target in the ventral hippocampus were at least 8/group (see Table 1).

The acute clozapine drug test sessions were run three times a week, with a day off between sessions. For 3 weeks, doses of clozapine (0, 1.25, and 2.5 mg/kg) were injected (s.c.) 20 min before testing in a volume of 1 ml/kg in a repeated measure counterbalanced design in which each dose was given to each animal once each week with the order counterbalanced. After the fourth week, the mini-pump infusions were stopped (see Table 2).

Histology

After all drug sessions were complete, each rat was anesthetized with sodium pentobarbital (100 mg/kg). A solution of saline and Chicago sky-blue dye was infused into each cannula, to help identify the placement of the cannulae. The rats were then perfused with a 9% phosphate buffered saline solution followed by 4% paraformaldehyde solution. The brains were removed and preserved in 4% formaldehyde. Before being sliced on a cryostat to make histological slides, the brains were frozen on dry ice. Histological slides were then made and studied under a microscope. Only those rats with both cannulae within the ventral hippocampus were included in the data analysis.

Data analysis

The choice accuracy (entries to repeat) and response latency (square root of seconds/entry) were assessed by a mixed between and within-subjects design analysis of variance with chronic systemic nicotine and chronic hippocampal nicotinic antagonist infusion as between subjects factors and acute systemic clozapine dose as a repeated measure. Significant interactions were followed up with analyses of the simple main effects to determine the sources of the significant interaction. An alpha value of $p < 0.05$ was used as the threshold for significance.

Results

All the animals were given each of the acute systemic doses of clozapine (0, 1.25, and 2.5 mg/kg) in a repeated measures counterbalanced order each week during weeks 2–4 of chronic drug administration. There were two between-subjects treatments; rats were given chronic local ventral hippocampal infusions of the nicotinic antagonists MLA or DH β E or the vehicle aCSF. The other between-subjects treatment was chronic systemic nicotine. Approximately half of the rats in each local infusion treatment group were given chronic systemic nicotine infusions while the others were given saline vehicle systemic infusions.

There was a differential effect of acute clozapine on memory in rats given each chronic hippocampal nicotinic antagonist infusion vs vehicle hippocampal infusions (Fig. 1). The interaction of chronic hippocampal nicotinic antagonist infusion $\times$ acute clozapine injection was significant ($F(4,140)=4.03$, $p < 0.005$) with regard to the choice accuracy measure, entries to repeat. In the rats given control vehicle aCSF hippocampal infusions, the 2.5 mg/kg clozapine dose caused a significant memory impairment ($p < 0.005$), whereas the lower clozapine dose of 1.25 mg/kg did not have a significant effect in this group. In the rats given chronic hippocampal DH β E infusions, there was a dramatic working memory impairment as indexed by decreased entries to repeat ($p < 0.001$) when no acute systemic clozapine challenge was given, only saline injection. This DH β E-induced memory impairment seen without clozapine treatment was attenuated by clozapine, with the 1.25 mg/kg clozapine dose causing a significant ($p < 0.025$) memory improvement. This was not due to a mere additivity of effects inasmuch as this dose of clozapine did not improve choice accuracy performance with hippocampal aCSF infusions. With the higher 2.5 mg/kg clozapine dose, the rats given chronic hippocampal DH β E showed a slight improvement relative to this group's performance after saline injection, but this was

not significant. In the rats given chronic hippocampal MLA, there was a different pattern of clozapine effects. The MLA dose tested (82 µg/side/day) was not found to impair memory performance in the absence of clozapine challenge (saline injection); however, this MLA dose did potentiate the amnesic effect of clozapine. In rats chronically infused with MLA, there were significant memory impairments with lower 1.25 mg/kg clozapine dose ($p<0.01$) as well as with the higher clozapine dose 2.5 mg/kg ($p<0.025$), whereas the rats given hippocampal aCSF infusions only showed a significant impairment with the higher 2.5 mg/kg clozapine dose. Direct comparison between aCSF- and MLA-treated rats in terms of memory performance showed that the MLA-treated rats were significantly ($p<0.05$) more impaired by 1.25 mg/kg of clozapine than the aCSF-treated rats. Chronic nicotine was not seen to have a significant main effect or interactions with regard to choice accuracy (Fig. 2).

With response latency, chronic nicotine infusion had a significant ($F(1,70)=4.11, p<0.05$) main effect of decreasing latency (Fig. 3). The rats not given nicotine averaged 48.5 ± 2.9 s/entry while those given chronic nicotine infusion averaged 40.5 ± 2.8 s/entry. There was also a significant ($F(2,140)=26.42, p<0.0001$) main effect of clozapine. Both the 1.25 mg/kg ($p<0.005$) and the 2.5 mg/kg ($p<0.0001$) clozapine doses caused significant increases in response latency relative to control. Chronic nicotinic antagonist administration into the ventral hippocampus did not significantly affect latency. There were no significant interactions of nicotine or clozapine with each other or nicotinic antagonist administration. Figure 3 is shown to compare the performance of the rats on the latency measure compared with the accuracy measure (Fig. 1). In contrast to the differential effects of clozapine in the different nicotinic antagonist groups with regard to choice accuracy, there was a very consistent clozapine effect on choice latency in these different nicotinic antagonist treatment groups.

Discussion

Clozapine effects on working memory critically depend on the integrity of hippocampal nicotinic receptors. Replicating our previous work (Addy and Levin 2002; Addy et al. 2005) clozapine caused a dose-dependent radial-arm maze working memory impairment in intact rats. In the current study, clozapine treatment had differential effects on working memory function in rats given chronic local hippocampal infusions of DHβE and MLA relative to control aCSF infusions. Nicotinic receptor subtypes in the hippocampus appear to play quite different roles in the manifestation of clozapine effects on memory.

Nicotinic $\alpha4\beta2$ blockade in the ventral hippocampus by chronic DHβE infusion caused a significant working memory impairment in the radial-arm maze, replicating our previous work (Arthur and Levin 2002). In the current study, the memory impairment caused by chronic hippocampal DHβE infusion was significantly attenuated by acute systemic administration of the antipsychotic drug clozapine. It is interesting to note that the effective dose of clozapine did not improve choice accuracy in the rats given vehicle hippocampal infusions. In fact, a higher dose impaired memory in subjects with vehicle hippocampal infusions.

The efficacy of clozapine in attenuating a working memory impairment caused by hippocampal dysfunction was seen previously in our research. Knife-cut lesions of the fimbria-fornix, which carries cholinergic innervation to the hippocampus among other fibers, caused a significant working memory impairment in the radial-arm maze, an effect which was significantly attenuated by clozapine (Addy et al. 2005). The current study provided a more specific challenge to the hippocampal cholinergic system, chronic blockade of $\alpha4\beta2$ nicotinic receptors. Inasmuch as this produced a similar effect as the fimbria-fornix lesion, it appears that ventral hippocampal $\alpha4\beta2$ receptor stimulation is a key link in the cascade of clozapine effects which underlies its cognitive impairing effects. Suppression of this receptor system from properly

functioning eliminated clozapine-induced impairment and instead permitted expression of its memory improving effects.

A different interaction was seen with regard to clozapine effects in the face of hippocampal $\alpha 7$ nicotinic blockade. Nicotinic $\alpha 7$ blockade in the ventral hippocampus by chronic MLA infusion dose used was not found to, by itself, significantly affect memory, but it did significantly affect response to clozapine. In contrast to the clozapine interactions with DH β E, clozapine-induced amnesic effects were potentiated by chronic hippocampal infusion of MLA. Ventral hippocampal nicotinic $\alpha 7$ receptors seemed to be key in protecting against the negative effects of clozapine on memory function. However, nicotinic $\alpha 7$ receptors are not the only receptors affected by MLA. Nicotinic $\alpha 6$ containing receptors, which also have been found to bind MLA (Zoli et al. 2002). These have been found to be important with regard to nicotinic actions on dopamine projections in the striatum (Zoli et al. 2002).

The differential effects of these nicotinic antagonists with clozapine appeared to be fairly specific to choice accuracy inasmuch as there was no apparent differential effect on clozapine-induced increased latency. It is interesting to note that chronic systemic nicotine infusion was not seen to significantly interact with clozapine effects on memory. This may have been related to the modest damage to the hippocampus from the local infusion cannulae. Our previous work has shown that even mild damage to the ventral hippocampus that does not in itself cause memory impairment does block the memory improvement seen with chronic systemic nicotine infusion (Levin et al. 1999). Even so, there was a modest improvement in the nicotine-treated rats when no other drug treatment was given.

Clozapine induced memory improvement in conditions of hippocampal dysfunction such as decreased $\alpha 4\beta 2$ receptor activity in the current study or fimbria-fornix lesions in our earlier study (Addy et al. 2005) suggests that clozapine may have better efficacy for cognitive improvement in patients with high affinity nicotinic receptor dysfunction in the hippocampus such as patients with Alzheimer's disease. The potentiated clozapine-induced memory impairment with hippocampal $\alpha 7$ receptor blockade suggests that clozapine may be less beneficial with regard to cognitive function in syndromes with hippocampal $\alpha 7$ deficits such as schizophrenia. However, there is evidence that clozapine effectively produces cognitive improvement in people with schizophrenia (Goldberg and Weinberger 1994; Meltzer and McGurk 1999). Areas other than the hippocampus may also be important for clozapine effects on cognitive function in schizophrenia. There may be heterogeneity within schizophrenia with those people with more pronounced $\alpha 7$ deficits in the hippocampus having less beneficial effects of clozapine.

Clozapine could be having direct effects on nicotinic receptors. Clozapine has been found to inhibit nicotine high affinity at binding nicotinic acetylcholine receptors (Park et al. 2001). Functionally, clozapine has been found to decrease some nicotine-related behaviors. Clozapine impairs the conditioned discriminative stimulus properties of nicotine (Brioni et al. 1994). Clozapine decreases smoking in patients with schizophrenia (McEvoy et al. 1995). However, these functional effects may be due to clozapine blockade of receptor systems such as dopaminergic or serotonergic receptors (Zhang and Bymaster 1999) downstream from nicotinic receptors as well as direct actions on nicotinic receptors.

This study provides important new information concerning the actions of clozapine on memory in the face of impaired neural function. Chronic blockade of hippocampal $\alpha 4\beta 2$ nicotinic receptors with DH β E impaired working memory in the radial-arm maze. Systemic administration of clozapine, which impaired memory in intact animals, significantly attenuated this impairment. This may have relevance for novel treatments of Alzheimer's disease and schizophrenia, which are characterized by both cognitive impairment and reductions of

nicotinic receptors in the hippocampus (Freedman et al. 1995; London et al. 1989; Nordberg 2001). Insofar as chronic inhibition of ventral hippocampal $\alpha 4\beta 2$ nicotinic receptors partially mimicked the loss of these receptors in Alzheimer's disease and schizophrenia, the current study suggests that some of the actions of clozapine may be useful avenues for developing new therapeutic treatments. However, the potentiated effect of clozapine in animals with chronic inhibition of ventral hippocampal $\alpha 7$ receptors suggests limitation of the clozapine-like therapy in the face of $\alpha 7$ nicotinic receptor loss which is seen in schizophrenia and Alzheimer's disease as well. Because clozapine is quite multifaceted in its mechanisms of effect, it is possible that its effect of attenuating the memory impairment caused by hippocampal $\alpha 4\beta 2$ blockade is due to a different mechanism of action than the potentiation of clozapine-induced memory impairment by blockade of ventral hippocampal $\alpha 7$ nicotinic receptors. Further research is needed to pick apart which actions of clozapine are critical for these effects.

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Chronic Hippocampal Nicotinic Antagonist Infusion and Acute Clozapine Effects on Memory in the Radial-Arm Maze

Fig. 1.

Clozapine caused a significant working memory impairment (entries to repeat), 100 μ g/side/day DH β E caused a significant memory impairment, which was attenuated by clozapine treatment. MLA at a dose of 82 μ g/side/day did not cause a significant working memory impairment by itself, but it did significantly potentiate the memory impairment caused by low-dose clozapine (1.25 mg/kg) exposure relative to the effect of this clozapine dose in rats with control aCSF hippocampal infusions (mean $\pm$ SEM). The data are collapsed across the systemic nicotine and saline treated groups

Chronic Hippocampal Nicotinic Antagonist Infusion and Chronic Systemic Nicotine Interactions with Acute Clozapine Effects on Memory in the Radial-Arm Maze

Fig. 2.

Chronic systemic nicotine administration did not significantly interact with the acute systemic clozapine effects on memory or the effects of chronic hippocampal nicotinic antagonist infusions (mean±SEM)

Fig. 3. Response latency (square root of seconds per arm entry) was increased by clozapine in a dose-dependent manner, seconds per arm entry (mean $\pm$ SEM)

Table 1

Group sizes for hippocampal nicotinic antagonist and systemic nicotine treatments

Systemic vehicle + hippocampal vehicle	N=19
Systemic vehicle + hippocampal MLA	N=11
Systemic vehicle + hippocampal DH β E	N=8
Systemic nicotine + hippocampal vehicle	N=18
Systemic nicotine + hippocampal MLA	N=9
Systemic nicotine + hippocampal DH β E	N=11

This numbers include only those subjects with correct bilateral ventral hippocampal drug infusion cannulae

Table 2

Timeline for drug treatments in the study

Period of chronic drug administration			
		Week 1	Weeks 2–4
Maze pretraining	→Surgery implanting cannulae and minipumps for chronic s.c. nicotine infusion and chronic intrahippocampal MLA and DH β E infusion	→Recovery	→Clozapine (0, 1.25, and 2.5 mg/kg); each dose once per week