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# **Chronic administration of haloperidol and olanzapine attenuates ketamine-induced brain metabolic activation<sup>#</sup>**

**Gary E. Duncan, Seiya Miyamoto, and Jeffrey Lieberman**

Department of Psychiatry, School of Medicine,  
University of North Carolina Chapel Hill, NC

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## **RUNNING TITLE PAGE**

**Running Title:** Chronic antipsychotics and ketamine-induced 2-DG uptake

**Correspondence:**

Gary E. Duncan, Ph.D.  
Department of Psychiatry  
CB # 7090  
University of North Carolina School of Medicine  
Chapel Hill, NC 27599-7250

Telephone: 919-966-8237, FAX: 919-966-1844, email: [gduncan@med.unc.edu](mailto:gduncan@med.unc.edu)

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Abbreviations: 2-DG, <sup>14</sup>C-2-deoxyglucose; PCP, phencyclidine; NMDA, N-methyl-D-aspartic acid.

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## **Abstract**

The fact that chronic administration of typical and atypical antipsychotic drugs is required for optimal therapeutic response suggests that drug-induced adaptive neurochemical changes contribute to their mechanism of action. In the present study, the effects of chronic and acute haloperidol and olanzapine were compared on ketamine-induced activation of select brain regions, as reflected by altered regional  $^{14}\text{C}$ -2-deoxyglucose (2-DG) uptake. Rats were injected once daily with haloperidol (1 mg/kg) or olanzapine (10 mg/kg) for 21 days and 20-24 hrs after the final injection were challenged with saline or ketamine (25 mg/kg). The washout period was used to test the effects of chronic drug treatment without the influence of acute drug administration. In vehicle treated rats, ketamine increased 2-DG uptake in select brain regions, including medial prefrontal cortex, nucleus accumbens, caudate-putamen, stratum lacunosum-moleculare of hippocampus and basolateral nucleus of the amygdala. This selective activation was attenuated by prior chronic treatment with both haloperidol and olanzapine. After acute treatment, olanzapine, but not haloperidol, blocked the ketamine induced activation of 2-DG uptake. These data suggest that both haloperidol and olanzapine can induce adaptive responses that counteract effects of ketamine. However, the differences observed in the acute effects of the two drugs in the ketamine challenge model suggest that different mechanisms could be responsible for their common chronic action of attenuating ketamine-induced brain metabolic activation.

## INTRODUCTION

Psychotomimetic effects of NMDA receptor antagonists in humans suggest that reduced NMDA receptor function may contribute to the pathophysiology of schizophrenia. Antagonists of the NMDA receptor such as ketamine and phencyclidine (PCP) induce a spectrum of behavioral effects that mimic positive, negative and cognitive symptoms of schizophrenia (Luby *et al.*, 1959; Cohen *et al.*, 1962; Krystal *et al.*, 1994; Malhotra *et al.*, 1996; Lahti *et al.*, 2001). Furthermore, in stabilized schizophrenic patients, ketamine can precipitates positive symptoms that are remarkably similar to those experienced during active phases of the patient's illness (Lahti *et al.*, 1995b; Lahti *et al.*, 1995a; Malhotra *et al.*, 1997). The human experience with NMDA antagonists provide the foundation for the NMDA receptor hypofunction hypothesis of schizophrenia (Coyle, 1996; Javitt and Zukin, 1991; Olney and Farber, 1995).

Preclinical studies of the effects of antipsychotic drugs in paradigms involving challenge with NMDA receptor antagonists provide support for the NMDA receptor hypofunction hypothesis. Extensive preclinical data demonstrate differential effects of acutely administered "typical" antipsychotics and "atypical" antipsychotic drugs on responses to NMDA antagonists. Although there is no universal consensus on precise definitions for "typical" and "atypical" antipsychotics, the typical drugs are generally considered those whose primary mechanism is D2 dopamine blockade (e.g. haloperidol, chlorpromazine). The atypical drugs are characterized by having more complex mechanisms of action that are not fully understood and demonstrate clinical efficacy with reduced side effects (e.g. clozapine, olanzapine). In a wide range of preclinical models some of the atypical drugs attenuate effects of NMDA antagonists but the typical drug do not. For example, clozapine and olanzapine, but not haloperidol or raclopride, attenuate the electrophysiological effects of PCP in brain slices (Wang and Liang, 1998;

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Arvanov *et al.*, 1997; Arvanov and Wang, 1999). Deficits in PPI and social behavior induced by NMDA antagonists (Bakshi *et al.*, 1994; Bakshi and Geyer, 1995; Corbett *et al.*, 1995) are attenuated by clozapine and olanzapine, but not by haloperidol and raclopride. However, Mansbuch *et al.* (2001) did find that haloperidol reduced PCP-induced deficits in PPI but to a lesser extent than did clozapine and ziprasidone. In studies of ketamine-induced brain metabolic activation, clozapine and olanzapine, but not haloperidol, blocked the activating effect of ketamine (Duncan *et al.*, 1998b; Duncan *et al.*, 2000). The well documented acute effects of atypical antipsychotics on responses to NMDA antagonists suggest that counteracting the effects of NMDA hypofunction could contribute to therapeutic mechanisms of action of the atypical drugs.

In addition to studying acute effects of antipsychotics, it is important to characterize neurobiological effects of chronic drug administration in preclinical models, since chronic treatment with antipsychotic drugs is essential for optimal therapeutic response. The therapeutically relevant adaptive changes induced by chronic antipsychotic drug treatments are poorly understood. Defining neurobiological adaptations induced by chronic drug exposure could provide insight into therapeutic mechanisms of currently used drugs and suggest novel treatment strategies that would mimic or promote adaptive responses.

In some preclinical models, quite different effects are found after chronic compared to acute antipsychotic administration. For example, acute treatment with clozapine and olanzapine enhanced NMDA-evoked electrophysiological responses but chronic treatment with the drugs reduced NMDA receptor sensitivity (Arvanov and Wang, 1999; Jardemark *et al.*, 2000). Also, chronic haloperidol was shown to reduce PCP-induced alterations in PPI under conditions where

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as acute administration of this typical antipsychotic did not affect PCP-induced PPI deficits (Pietraszek and Ossowska, 1998; Martinez *et al.*, 2000).

In our previous studies of the effects of antipsychotics on ketamine-induced alterations in regional brain metabolism, acute effects of the antipsychotics were examined (Duncan *et al.*, 2000; Duncan *et al.*, 1998b). The present investigation characterized the effects of chronic administration of haloperidol and olanzapine in the ketamine challenge-metabolic mapping paradigm.

## MATERIALS AND METHODS

### Animals and treatments

Sprague-Dawley rats (Harlan Laboratories) were housed under a 12 hr light-dark cycle with lights on at 0700 hrs and had continuous access to food and water. All procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina Institutional Animal Care Committee.

For chronic studies, rats weighing initially 150-175 grams were injected i.p. once daily for 21 days with vehicle (10  $\mu$ l 20% acetic acid/ml of 0.9% saline), haloperidol (1 mg/kg), or olanzapine (10 mg/kg). After 21 days of treatment, the rats in vehicle, haloperidol, and olanzapine groups weighed respectively (grams, mean  $\pm$  SEM)  $300 \pm 7$ ,  $282 \pm 7$ , and  $253 \pm 5$ . The 2-DG uptake assessments were made 20-24 hrs after the final injection in the series (see below). For acute studies, rats were injected i.p. with haloperidol (0.5 mg/kg), olanzapine (10 mg/kg) or vehicle 1 hr before the 2-DG. These doses were chosen for the acute studies because preliminary experiments found that they were the minimal doses required to completely block the behavioral activation induced by ketamine. Acute administration of 0.5 mg/kg haloperidol produced catalepsy in 100% of rats.

Three to five days before the 2-DG injections, jugular catheters were implanted under pentobarbital anesthesia and exteriorized at the base of the neck, so the 2-DG could be administered i.v. with minimal stress on the day of the experiment. After surgery, catheters were flushed daily with 0.9% saline to acclimate rats to the handling involved in the experimental procedures. Rats were transported from the animal quarters to the laboratory 4-6 hours before

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initiation of the 2-DG experiment and the 2-DG was given 20-24 hrs after the final injection of antipsychotics or vehicle.

### **High resolution autoradiographic analysis of $^{14}\text{C}$ -2-DG uptake**

The high-resolution autoradiographic procedures for analysis of [ $^{14}\text{C}$ ]-2-deoxyglucose (2-DG) uptake have been described in detail (Duncan *et al.*, 1993; Duncan *et al.*, 1998a; Duncan *et al.*, 2000). Behavioral activation was evident within 2 min after ketamine injection in vehicle pretreated rats and the 2-DG (American Radiolabeled Chemicals, 300 mCi/mmol, 0.4  $\mu\text{Ci/g}$  body weight) was administered via the jugular catheter 3 min after i.p. injection of ketamine or saline. Rats were killed by decapitation 5 min after the i.v. injection of 2-DG to ensure a constant behavioral state during the 2-DG uptake period. We have demonstrated that a 5 min survival period after i.v. injection of 2-DG is useful for the study of time-limited behavioral events (Duncan *et al.*, 1993; Duncan *et al.*, 1998b; Duncan *et al.*, 1998a; Duncan *et al.*, 2000). Brains were frozen on an aluminum block cooled with liquid nitrogen and stored at  $-80\text{ }^{\circ}\text{C}$  until sectioned. Kodak SR Industrex film was cut into rectangular pieces approximately the 3/4 the length of microscope slides and glued to one end of the slides with Silicone adhesive. Cryostat sections (10  $\mu\text{m}$ ) of the brains were thaw-mounted onto the slide-mounted film under safe-light conditions and stored in light-tight desiccator boxes at room temperature for exposure periods of 6 weeks. The autoradiograms produced by thaw mounting sections onto the high-resolution film were used for photographic documentation of 2-DG uptake patterns. For quantitative analysis, other sections were mounted onto microscope slides and apposed to Kodak Industrial T film in x-ray cassettes, along with  $^{14}\text{C}$  microscale standards (Amersham) for 2 weeks.

Autoradiograms were digitized with a high-resolution transparency scanner (Linotype-Hell, Saphir Ultra, Happauge NY) and analyzed with NIH Image software. Thirteen brain



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regions were chosen for quantitative evaluation based on our previous investigations of the effects of ketamine and antipsychotic drugs on 2-DG uptake. The regions chosen for study were previously shown to exhibit ketamine-induced increases in 2-DG uptake [medial prefrontal cortex (prelimbic cortex of Paxinos and Watson, 1997), anterior cingulate cortex, retrosplenial cortex, nucleus accumbens, caudate putamen, basolateral amygdala, and the dentate molecular layer and stratum lacunosum-moleculare of the hippocampus, as well as “control” regions [lateral frontal cortex (somatosensory cortex), medial septum, ventromedial hypothalamus, CA3 and CA1 stratum radiatum] where no effects of ketamine were expected. Each of the 13 brain regions was analyzed in 4 sections for each animal and each treatment condition by observers blind to treatment conditions.

### **Statistics**

PC-based SYSTAT software (version 9.0; SPSS, Chicago IL) was used for statistical analysis. A separate analysis of variance (ANOVA) was performed for each brain region for the three separate experiments of the study (i.e. chronic olanzapine and haloperidol, acute olanzapine, and acute haloperidol). Where significant effects were indicated in the ANOVA ( $p < .05$ ), a set of planned comparisons was made by Tukey tests. The specific planned comparisons were chosen to assess whether the antipsychotic drugs alone altered 2-DG uptake, or whether they altered the effects of ketamine on 2-DG uptake.

## RESULTS

### **Behavioral and brain metabolic responses to ketamine after chronic haloperidol and olanzapine**

Rats were tested 20-24 hr after the final injection in the chronic series. After saline injection, there was no obvious difference in the behavior of rats treated chronically with haloperidol or olanzapine compared to controls. In all vehicle-treated rats, ketamine (25 mg/kg) induced a typical behavioral response of staggered locomotion and repetitive stereotypic side-to-side head movements. For rats in the chronic olanzapine and haloperidol groups, the behavioral activation induced by ketamine was reduced. After olanzapine and haloperidol, respectively, 5/9 and 7/9 rats exhibited no ketamine-induced behavioral activation as defined by staggered locomotion and stereotypic head movements. For the rats in the chronic olanzapine and haloperidol groups that did show a response to ketamine, the behavioral activation was noticeably less vigorous in comparison to the rats injected chronically with vehicle and challenged with ketamine.

Ketamine induced a neuroanatomically selective activation that was significantly reduced by chronic treatment with haloperidol and olanzapine. Autoradiograms of rats in the different treatment groups are shown in Figure 1 and quantitative data are in Figure 2. In vehicle-treated rats, ketamine induced a robust activation of 2-DG uptake in the medial prefrontal cortex, anterior cingulate cortex, retrosplenial cortex, nucleus accumbens, caudate-putamen, basolateral nucleus of the amygdala, and stratum lacunosum-moleculare of the hippocampus. Chronic treatment with both haloperidol and olanzapine significantly reduced the ketamine-induced activation in all of these regions except for the nucleus accumbens. For the accumbens there was a trend for both haloperidol and olanzapine to reduce the effects of ketamine but the differences

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were not significant at the  $p < .05$  level. In regions that did not show increased 2-DG uptake in response to ketamine, the antipsychotics did not alter 2-DG uptake in comparison to the vehicle-injected controls.

### **Behavioral and brain metabolic responses to ketamine after acute haloperidol and olanzapine**

In the rats injected acutely with haloperidol (0.5 mg/kg) or olanzapine (10 mg/kg) and then injected with saline one hour later, increased 2-DG uptake was observed in the lateral habenula and decreased relative uptake observed in the retrosplenial cortex (Figs 3 and 4). Other regions assessed did not show significant differences in relative 2-DG uptake. Administration of both haloperidol and olanzapine, one hr before ketamine, completely blocked ketamine-induced behavioral activation in all rats. In contrast to the similar effects of the two drugs on ketamine-induced behavioral activation, only olanzapine blocked ketamine-induced brain metabolic activation (Figs 2 and 3).

## Discussion

Subanesthetic doses of ketamine induce marked alterations in regional patterns of brain metabolism. As observed in our previous studies with rats (Duncan *et al.* 1998a,b; 2000) and mice (Miyamoto *et al.*, 2000; Miyamoto *et al.*, 2001; Duncan *et al.*, 2002) the present work found selective ketamine-induced activation of the limbic cortical regions, sub-regions of the hippocampus, nucleus accumbens, caudate-putamen, and basolateral amygdala. Chronic administration of haloperidol and olanzapine attenuated the brain metabolic activation induced by ketamine. The mechanisms for NMDA antagonist-induced brain metabolic and behavioral activation are poorly understood but may paradoxically involve glutamatergic activation. Subanesthetic doses of NMDA antagonists increase brain extracellular fluid levels of glutamate (Bustos *et al.*, 1992; Moghaddam *et al.*, 1997), presumably by disinhibitory mechanisms (Farber *et al.*, 2002; Greene, 2001, Olney and Farber, 1995). These excitatory amino acids could then activate non-NMDA receptors (Moghaddam *et al.*, 1997) as well as NMDA receptors unblocked by low doses of ketamine. In contrast to the increase in glutamate release by sub-anesthetic doses of ketamine, anesthetic doses of the drug decrease glutamate levels (Moghaddam *et al.*, 1997). The effects of different doses of ketamine on excitatory amino acid levels are consistent with our observations of increased 2-DG uptake in response to a subanesthetic dose, and global reduction in 2-DG uptake in response to an anesthetic dose of ketamine (Duncan *et al.*, 1998a). Whether chronic antipsychotic administration enhances NMDA receptor function or attenuates the effects of ketamine by modulating glutamate or other neurotransmitter systems will require further study. The acute actions of antipsychotic drugs to counteract effects of NMDA antagonists do not apparently involve reduced presynaptic glutamate release in the medial prefrontal cortex of

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the rat, since the drugs did not affect PCP induced glutamate releases as assessed by microdialysis (Adams and Moghaddam, 2001). However, there are no data available for the effects of chronic antipsychotic drug treatment on NMDA antagonists-induced release of glutamate.

In the present work, one purpose of using a 20-24 hr washout period after the final injection in the chronic series was to minimize the acute pharmacological actions of olanzapine and haloperidol at the time of ketamine challenge. Both drugs produce sedative effects after acute administration that could confound interpretation of results. The plasma half-life of both clozapine and haloperidol in rats is approximately 1.5 hrs (Cheng and Paalzow, 1992; Baldessarini *et al.*, 1993). Therefore, 24 hrs after the final injection, there should be minimal circulating antipsychotic drug at the time of the ketamine challenge. There were no apparent behavioral effects of the drugs in rats 20-24 hrs after the final drug injection under control conditions. Also, the characteristic activation of the lateral habenula after acute haloperidol and olanzapine treatment was not seen after the washout period. However, the behavioral and brain metabolic responses to ketamine challenge were attenuated 20-24 hrs after the last injection of both haloperidol and olanzapine. These data suggest that adaptive changes were induced by both drugs to counteract the effects of ketamine.

In many model systems based on acute effects of antipsychotic drugs, treatment with haloperidol is ineffective or less effective than clozapine and other atypical antipsychotics in reversing effects of NMDA antagonists (see Introduction). However, in studies of PCP-induced deficits in PPI, chronic administration of haloperidol antagonized effects of PCP (Ossowska *et al.*, 2000; Pietraszek and Ossowska, 1998). Thus after chronic administration, haloperidol can block disruptive behavioral effects of NMDA antagonists. Such findings are similar to results of

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the present investigation showing that chronic (but not acute) administration of haloperidol attenuated effects of ketamine. Since acute administration of olanzapine reduced ketamine-induced brain metabolic activation but acute treatment with haloperidol did not, it is possible that the effects of the two drugs observed after chronic treatment could involve different mechanisms.

In contrast to selective attenuation of PCP-induced PPI deficits by atypical antipsychotics, amphetamine-induced disruption of PPI is consistently reported to be reduced by acute administration of both typical and atypical antipsychotic drugs (for review see Geyer *et al.*, 2001). However, after chronic administration, neither the typical or atypical antipsychotics affect the altered sensory gating induced by amphetamine (Andersen and Pouzet, 2001). As noted above, chronic administration of both typical and atypical antipsychotics attenuated PCP-induced deficits in PPI. Thus, in the PPI model of sensory gating, adaptive changes induced by chronic antipsychotic treatments appear to attenuate effects of NMDA antagonists but not effects of amphetamine.

Mechanisms responsible for the observed effects of chronic haloperidol and olanzapine on responses to NMDA antagonists could involve alterations in postsynaptic glutamate receptors. Administration of both typical and atypical antipsychotic drugs can alter glutamate receptor binding and expression of specific subunits of the receptor (see Table 1). A detailed discussion of the complex results of those studies is beyond the scope of the present paper but major findings are summarized in Table 1. Both increases and decreases in binding sites and subunit expression have been reported. It is not known whether such changes reflect increased or decreased function of glutamate receptors.

Studies that examined functional responses to NMDA after chronic antipsychotic treatments suggest that both typical and atypical drugs induce an adaptive reduction in NMDA

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receptor sensitivity. Ossowska (1995) found that chronic administration of haloperidol attenuated turning behavior produced by microinjection of NMDA into the caudate-putamen. Also, Jardemark *et al.* (2000) found that 21 day treatment with haloperidol, clozapine, and olanzapine reduced the electrophysiological sensitivity to NMDA in slices of the medial prefrontal cortex. The treatment duration and washout period (24 hrs) in the study by Jardemark *et al.* (2000) were the same as those used in the present report. One interpretation of the results of the present study is that reduced NMDA receptor function, in circuits responsible for ketamine-induced metabolic activation, is related to the attenuated response to ketamine observed after chronic haloperidol and olanzapine treatments.

As noted previously, it may seem paradoxical that reduced NMDA receptor function could counteract effects of an NMDA receptor antagonist. However, the neuroanatomically specific brain metabolic response to ketamine apparently involves activation of glutamatergic neurotransmission, since glutamate provides the primary excitatory drive in the brain. It will be of interest in future studies to determine whether the attenuation of the effects of NMDA antagonists by chronic administration of typical and atypical antipsychotic drugs involves molecular alterations in NMDA receptors in specific cell types and circuits, or altered monoamine-glutamate interactions.

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**Footnotes**

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**Send reprint requests to:**

Gary E. Duncan, Ph.D.  
Department of Psychiatry  
CB # 7090  
University of North Carolina School of Medicine  
Chapel Hill, NC 27599-7250



## Legends to Figures

Figure 1. Autoradiographic images of 2-DG uptake. Rats were injected with olanzapine (10 mg/kg) or haloperidol (1 mg/kg) once daily for 21 days and challenged with saline or ketamine 20-24 after the final injection. The first treatment designation above the images refers to chronic treatment and the second refers to the acute challenge. II/IV- layers 3 and 4 of the somatosensory cortex. For other abbreviations see legend to Figure 2.

Figure 2. Effects of chronic olanzapine and haloperidol on ketamine-induced changes in 2-DG uptake. Data in this and subsequent figures are ratios of radioactivity in the regions of interest to that of the CA1 stratum radiatum of the hippocampus (means  $\pm$  SEM). The ANOVAs for each experiment indicated no significant group effect in absolute 2-DG uptake for the CA1 region any experiment ( $p > .3$  for each of the 3 separate experiments for which data is shown in Figures 2-4). Abbreviations: MFC, medial prefrontal cortex (prelimbic cortex of Paxinos and Watson, 1997), LFC lateral frontal cortex (primary somatosensory cortex); Cing, anterior cingulate cortex; MS, medial septum; Cpu, caudate putamen; Acb, nucleus accumbens; BL basolateral nucleus of amygdala; SLM, stratum lacunosum-moleculare of hippocampus; CA3 SR, cornu Ammonis region 3 of the stratum radiatum; DG, molecular layer of the dentate gyrus; Rspl, retrosplenial cortex; Lhab, lateral habenula. \* $p < .05$  compared to olanzapine-ketamine and haloperidol-ketamine by Tukey tests.

Figure 3. Effects of acute administration of olanzapine on ketamine-induced 2-DG uptake. Rats were injected with olanzapine (10 mg/kg) 1 hour before challenge with ketamine.

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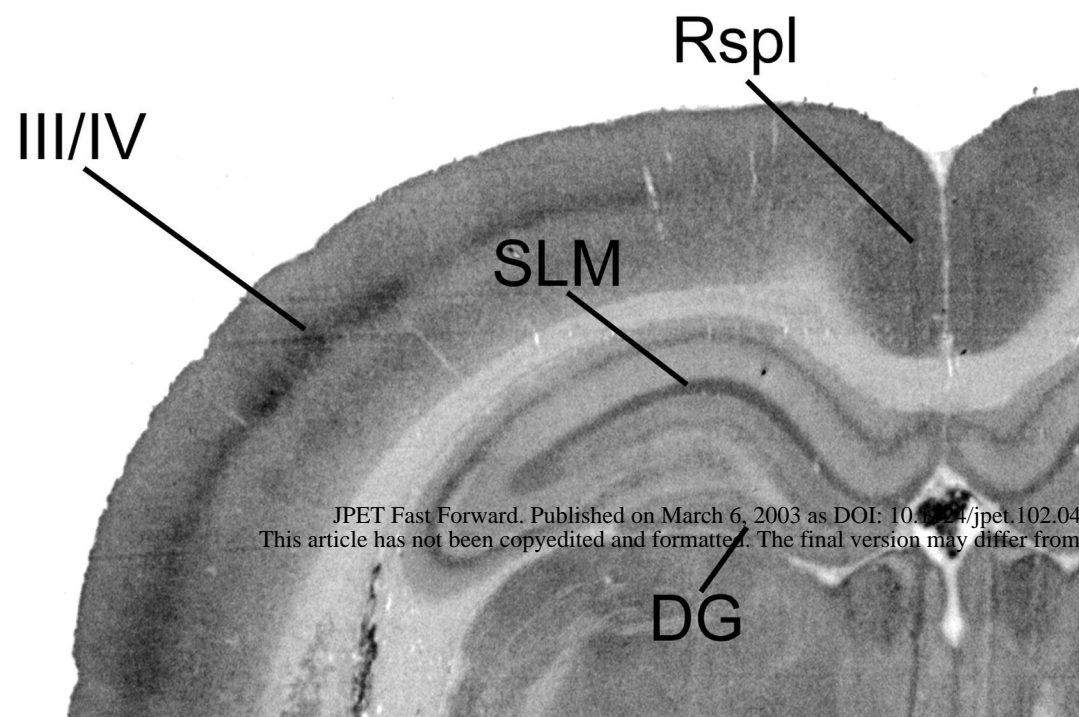
See Figure 1 for abbreviations. \* $p < .05$  compared to olanzapine-ketamine. \*\* $p < .01$  compared to saline-saline.

Figure 4. Lack of effect of acute haloperidol on ketamine-induced 2-DG uptake. Rats were injected with haloperidol (0.5 mg/kg) 1 hour before challenge with ketamine. See Figure 1 for abbreviations. \*  $p < .05$  compared to saline-saline. \*\* $p < .05$  compared to saline-saline (for haloperidol-saline) and compared to saline-ketamine (for haloperidol-ketamine).

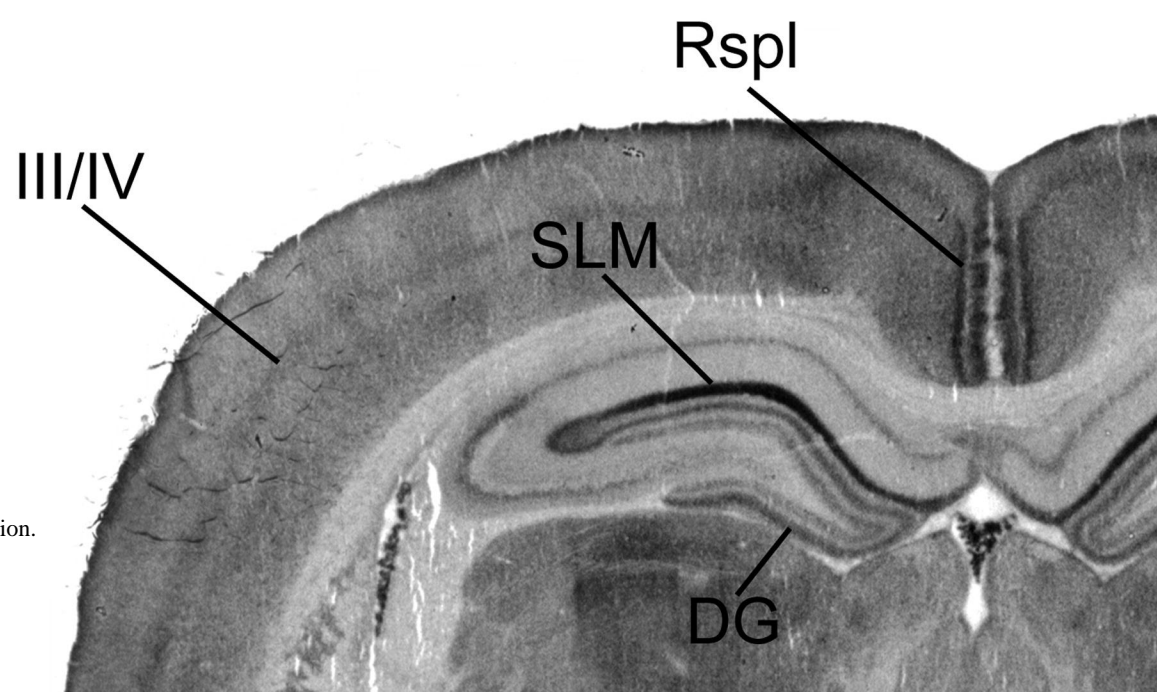
**Table 1**

<b>Major findings in studies examining effects of chronic antipsychotic drug treatments on glutamate receptor binding and mRNA expression.</b>	
<b>Study</b>	<b>Major Effects After Chronic Treatment</b>
(Tascedda <i>et al.</i> , 2001)	Olanzapine increased AMPA mRNA (GluB and GluC ) in hippocampus. No effect on NMDA, or metabotropic receptor subunits message in hippocampus. In striatum, no effect of olanzapine on NMDA, AMPA or metabotropic mRNA.
(Chen and Gurling, 1999)	Flupenthixol did not alter mRNA levels of Glu1-7, KA1, or KA2.
(Ossowska <i>et al.</i> , 1999)	Clozapine and haloperidol had no effect on MK801 binding but both increased <sup>3</sup> H-CGP-39653 (competitive NMDA antagonist) binding in cortex.
(Tascedda <i>et al.</i> , 1999),	Clozapine decreased GluC in Acb. No effect of haloperidol apparent.
(Giardino <i>et al.</i> , 1997)	Clozapine but not haloperidol decreased MK801 binding in cortex
(Tarazi <i>et al.</i> , 1996)	Clozapine and haloperidol decreased MK801 binding in cortex. Clozapine but not Haloperidol decreased MK801 binding caudate.
(Riva <i>et al.</i> , 1997)	Haloperidol but not clozapine increased NR1 and NR2A-C mRNA in Striatum. Clozapine but not haloperidol decreased NR2C in cortex and accumbens.
(Fitzgerald <i>et al.</i> , 1995)	Haloperidol, but not clozapine, increased NR1 protein in striatum. Both haloperidol and clozapine increased GluR1 in cortex..
(Ulas <i>et al.</i> , 1993)	Haloperidol increased NMDA binding in cortex but not hippocampus.

# Vehicle/Saline



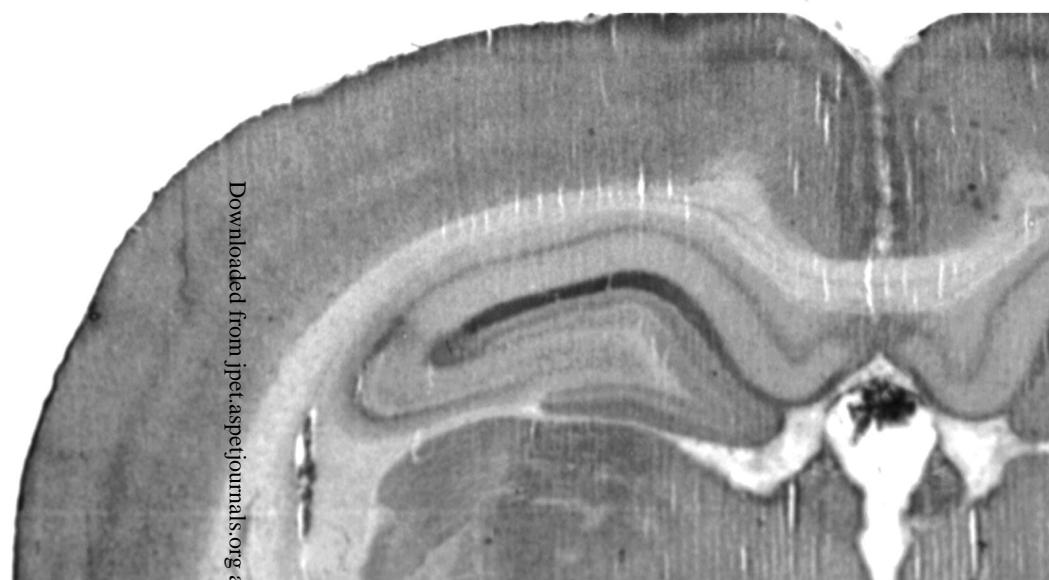
# Vehicle/Ketamine



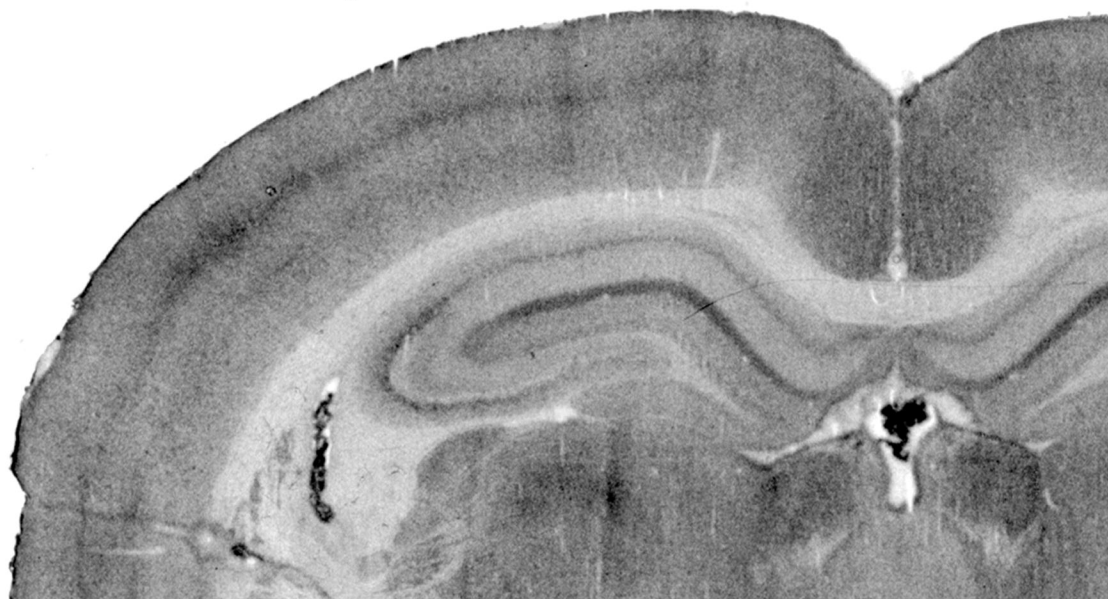
# Olanzapine/Saline



# Olanzapine/Ketamine



# Haloperidol/Saline



# Haloperidol/Ketamine



