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Differential effects of NMDA receptor antagonism on spine density

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Dendritic spines are the postsynaptic structures for excitatory synaptic transmission. The number, or density, of spines along a dendrite is tightly regulated, and numerous studies have demonstrated that even a modest increase or reduction in spine number alters neuron physiology and is associated with changes in behaviour (Pérez-Vega et al., 2000, Knafo et al., 2001, Knafo et al., 2005, Kolb et al., 2008, Roberts et al., 2009, Lai et al., 2012, Sanders et al., 2012).

One association that has been made between spine density and behaviour concerns the psychiatric condition major depression. Postmortem studies have detected reductions in spine density in patients with major depressive disorder (Penzes et al., 2011, Kang et al., 2012). Additionally, a rodent model of depression showed deficits in cortical spine density when exposed to chronic, unpredictable stress (Li et al., 2011). One study attributed the antidepressant effects of ketamine, a non-competitive NMDA receptor (NMDAR) antagonist, in part to the ability of this compound to increase dendritic spines in rats (Li et al., 2010). The finding that acute ketamine treatment leads to increased spine density in the cortex in rats is surprising, given that several studies, including one from our laboratory, have consistently reported that NMDAR antagonism with MK-801 or phencyclidine reduces spine density in both the cortex and the striatum (Butler et al., 1999, Elsworth et al., 2011, Ramsey et al., 2011, Velázquez-Zamora et al., 2011). In addition, many genetic models of reduced NMDAR levels and function show decreases in spine density in several brain regions (Das et al., 1998, Alvarez et al., 2007, Ulanir et al., 2007, Roberts et al., 2009, Brigman et al., 2010, Ramsey et al., 2011).

We hypothesized that there may be differential effects on spine density if NMDAR blockade is transient, as with ketamine, instead of sustained, as would be the case with MK-801 treatment. Our hypothesis was based on the different pharmacological actions of these drugs at the NMDAR, since ketamine has a much faster off-rate compared to MK-801: 0.2 and 0.003 s⁻¹ respectively (MacDonald et al., 1991). In addition, MK-801 has a higher selectivity for the NMDAR than ketamine, which has lower affinity interactions with other glutamate receptors and with cholinergic and opioid receptors (Kohrs and Durieux, 1998). Therefore, in this study, we compared ketamine and MK-801 with regards to their in vivo effects on spine density. Doses of ketamine and MK-801 were selected based on reported

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effects of 10 mg/kg ketamine on spine density (Li et al., 2010). We compared the effects of this dose with a higher dose of ketamine (20 mg/kg), and a dose of MK-801 that was equipotent with respect to NMDAR antagonism. We equated the 20 mg/kg of ketamine to 0.2 mg/kg dose of MK-801 based on their similar effects on locomotor activity (Tricklebank et al., 1989). We studied the effects of acute treatment and repeated daily injections on layer V pyramidal neurons in the medial frontal cortex and medium spiny neurons in the dorsal striatum of wild-type mice. All experiments were performed in accordance with Canadian Council on Animal Care guidelines and approved by the University of Toronto Animal Care Committee. Male C57BL/6 mice aged 11–13 weeks were purchased from Charles River Laboratories. For acute treatment, animals received a single i.p. injection of saline, 10 mg/kg, or 20 mg/kg ketamine hydrochloride (Toronto Research Chemicals) or 0.2 mg/kg MK-801 (Sigma-Aldrich) dissolved in saline. For subchronic treatment, animals received daily i.p. injections of saline, ketamine (10 or 20 mg/kg) or MK-801 (0.2 mg/kg) for seven consecutive days. To ensure consistency, experiments were performed in groups based on the drug treatment. For all ketamine studies, injections of saline, 10 mg/kg ketamine and 20 mg/kg ketamine were performed at the same time. For all MK-801 studies, injections of saline and 0.2 mg/kg MK-801 were performed at the same time.

Twenty-four hours after the last injection, animals were transcardially perfused with phosphate buffered saline then with 4% paraformaldehyde. Vibratome sagittal sections 150 μ m thick were prepared and neurons were randomly labeled by diolistic labeling as described previously (O'Brien and Lummis, 2006, Ramsey et al., 2011). DiI labeled sections were subsequently processed for immunofluorescence to identify layer V pyramidal neurons using a monoclonal Neurofilament 200 (N200) antibody (1:200 dilution, Sigma-Aldrich). Neurons were imaged using Olympus Fluoview FV 1000 software with IX81 confocal microscope at 60X magnification and 0.5 μ m step Z-stack images of dendrites were collected. For each treatment group, multiple dendrites (2–6) were imaged for each animal, with the criterion that the dendrite images are taken from a region between 20 μ m and 50 μ m from the cell soma. Dendrite images from cortical pyramidal neurons were taken exclusively from the basolateral dendrites. Spine density was determined using maximum projection images and Nikon NIS Elements software. The spine density was averaged from several dendrite images to generate a single spine density value for each animal. The (n) value presented in the results represents the number of animals that were examined from separate experiments. Measurements of spine length, head diameter, and neck diameter were performed on maximum projection images with NIH ImageJ using the NeuronJ plug-in (data not shown). Comparisons between saline- and ketamine-treated groups were performed using one-way ANOVA with post-hoc Bonferroni comparisons between saline and each drug treatment. Comparisons between saline- and MK-801-treated groups were performed using a paired Student's t-test, where observations were paired by brain region. Statistical significance was determined by a p value < 0.05.

Our studies confirmed that an acute, low dose of ketamine significantly increased dendritic spine density in layer V pyramidal neurons of the medial frontal cortex (Figure 1), the same region that was studied in the Li et al report. Interestingly, a higher dose of ketamine did not substantially increase spine density and acute ketamine treatment showed no significant

effect on spine density in the striatum. Acute MK-801 administration had no significant effect on cortical or striatal spine density.

The increase in spine density observed with acute ketamine was no longer seen with subchronic treatment. Instead, subchronic ketamine dose-dependently reduced cortical spine density (Figure 2). In the striatum, subchronic ketamine treatment at a dose of 10 mg/kg also reduced spine density. Furthermore, subchronic MK-801 treatment led to reductions in spine density in both the cortex and striatum (Figure 2). In addition, after morphological analyses of the dendritic spines, there was no significant change in spine length, head diameter or head/neck ratio in any treatment group (data not shown).

In summary, we report that ketamine and MK-801 have differential effects on spine density depending on the dose, regimen, and brain region. While acute ketamine does indeed increase cortical spine density, it has no effect in the striatum, and a decrease in spine density is observed with repeated treatment. Acute MK-801 treatment has no effect on cortical or striatal spine density, whereas repeated MK-801 reduces spine density in both cortex and striatum. These results are consistent with the notion that brief NMDAR antagonism, either due to acute exposure or fast off-rate, promotes cortical spinogenesis, while more sustained NMDAR antagonism leads to reductions in spine density.

Clinical and rodent studies have demonstrated antidepressant effects with a single, acute, low dose of ketamine (Berman et al., 2000, Zarate et al., 2006, Maeng et al., 2008, Li et al., 2010, Autry et al., 2011, Yang et al., 2012). This represents a significant advancement in the treatment of depression. However, our results suggest that repeated ketamine exposure may have detrimental effects on spine density over time. Consistent with this, repeated recreational ketamine use causes impairments in cognition and affect (Curran and Monaghan, 2001), and spine deficits have been correlated with cognitive deficits (Leuner et al., 2003, Xu et al., 2009, Yang et al., 2009, 2010). Our results also suggest that NMDAR antagonism has differential effects on the cortex and striatum. This could be due to the inherent differences between the neuronal cell types, including the fact that medium spiny neurons receive both glutamatergic and dopaminergic inputs, whereas the basolateral dendrites of layer V pyramidal neurons receive primarily glutamatergic inputs from layer III pyramidal neurons (Purves, 2001, Thomson and Bannister, 2003). In addition, medium spiny neurons receive afferent projections from layer V pyramidal neurons. Therefore, striatal effects of these drugs could be compounded by alterations in cortical physiology. Our study also demonstrates that different NMDAR antagonists have different effects on spine density; therefore, reductions in spine density may also occur with other NMDAR antagonists, and the therapeutic safety margin for depression treatment may therefore be limited. Consideration of pharmacological properties, including but not limited to dissociation rate and binding affinity, may be useful to guide the development of other NMDAR antagonists as alternatives to ketamine for the treatment of depression.

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Figure 1. Acute 10mg/kg ketamine treatment increases cortical spine density, while acute MK-801 treatment has no significant effect on spine density

A) Left panel: Quantification of spine density counts in the cortex 24 hours following acute i.p. injection of saline (SAL), 10 mg/kg ketamine (K10), 20 mg/kg ketamine (K20) or 0.2 mg/kg MK-801 (MK). One-way ANOVA for the effect of ketamine, $F=4.084$, $p=0.0384$. Bonferroni-corrected post hoc comparing SAL to K10, $t_2=2.806$ ($p<0.05$) and comparing SAL to K20, $t_2=1.874$ ($p>0.05$). Paired t-test for the effect of MK801, $p=0.4822$, $t_5=1.113$. Right panel: Representative images of basolateral dendrites from cortical layer V pyramidal neurons following saline or drug treatment.

B) Left panel: Quantification of spine density counts in the striatum 24 hours following acute i.p. injection of saline (SAL), 10 mg/kg ketamine (K10), 20 mg/kg ketamine (K20) or 0.2 mg/kg MK-801 (MK). One-way ANOVA effect of ketamine in striatum, $F=0.9121$, $p=0.4228$. Paired t-test for the effect of MK801, $p=0.7062$, $t_5=0.3993$. Right panel: Representative images of striatal medium spiny neurons following saline or drug treatment. For A and B, $*p<0.05$, (n) is indicated in bar, microphotograph scale bar = 5 microns.

Figure 2. Subchronic ketamine and MK-801 treatment reduce cortical and striatal spine density

A) Left panel: Quantification of spine density counts in the cortex 24 hours following a week of daily i.p. injections of saline (SAL), 10 mg/kg ketamine (K10), 20 mg/kg ketamine (K20) or 0.2 mg/kg MK-801. One-way ANOVA for the effect of ketamine, $F=4.086$, $p=0.0344$. Bonferroni-corrected post hoc comparing SAL to K10, $t_2=1.946$ ($p>0.05$) and comparing SAL to K20 $t_2=2.786$ ($p<0.05$). Paired t-test for effect of MK801, $p=0.0069$, $t_7=3.779$. Right panel: Representative images of basolateral dendrites from cortical layer V pyramidal neurons following saline or drug treatment.

B) Left panel: Quantification of spine density counts in the striatum 24 hours following a week of daily i.p. injections of saline (SAL), 10 mg/kg ketamine (K10), 20 mg/kg ketamine (K20) or 0.2 mg/kg MK-801. One-way ANOVA for the effect of ketamine, $F=3.388$, $p=0.0611$. Bonferroni-corrected post hoc comparing SAL to K10, $t_2=2.499$ ($p<0.05$) and comparing SAL to K20 $t_2=0.6171$ ($p>0.05$). Paired t-test for effect of MK801, $p=0.0157$, $t_7=3.171$. Right panel: Representative images of striatal medium spiny neurons following saline or drug treatment. For A and B * $p<0.05$ and ** $p<0.01$, (n) is indicated in bar, and microphotograph scale bar = 5 microns.