Temporally dissociable effects of ketamine on neuronal discharge and gamma oscillations in rat thalamo-cortical networks

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[#]The last two authors contributed equally to the study.

Short title: Ketamine and thalamo-cortical networks

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Abstract

Background: Sub-anesthetic doses of the non-competitive *N*-methyl-*D*-aspartate receptor (NMDA-R) antagonist ketamine evoke immediate and persistent antidepressant effects in treatment-resistant depressed patients and antidepressant-like effects in rodents through still poorly understood mechanisms. Since phencyclidine (PCP) disinhibits thalamo-cortical networks by blocking NMDA-Rs on GABAergic neurons of the reticular thalamic nucleus (RtN), we examined ketamine's actions in the same areas.

Methods: Single units and local field potentials were recorded in chloral hydrate anesthetized male Wistar rats. The effects of cumulative ketamine doses (0.25-5 mg/kg, i.v.) on neuronal discharge and oscillatory activity were examined in RtN, mediodorsal and centromedial (MD/CM) thalamic nuclei, and layer VI of the medial prefrontal cortex (mPFC).

Results: Ketamine (1, 2 and 5 mg/kg, i.v.) significantly decreased the discharge of MD/CM, RtN and layer VI mPFC pyramidal neurons. Simultaneously, ketamine decreased the power of low frequency oscillations in all areas examined and increased gamma oscillations in mPFC and MD/CM. Lower ketamine doses (0.25 and 0.5 mg/kg, i.v.) were ineffective.

Conclusions: As observed for PCP, ketamine markedly inhibited the activity of RtN neurons. However, unlike PCP, this effect did not translate into a disinhibition of MD/CM and mPFC excitatory neurons, possibly due to a more potent and simultaneous blockade of NMDA-Rs by ketamine in MD/CM and mPFC neurons. Hence, in contrast to the presumed excitatory action of ketamine via AMPA-R receptors following NMDA-R blockade, the present *in vivo* results show an early transient inhibition of neuronal discharge and an increase in gamma oscillations, an effect that may underlie its antidepressant action.

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Abbreviations:

(RM)-ANOVA, (repeated measures) analysis of variance

ACC, anterior cingulate cortex

AMPA-R, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors

AP, anterior posterior

CM, centromedial nucleus of the thalamus

FFT, fast Fourier transformation

GABA, gamma-Aminobutyric acid

HNK, (2R,6R)-hydroxynorketamine

IL, infralimbic cortex

i.p., intraperitoneal

i.v., intravenous

Ket, (R,S)-ketamine

L, lateral

LFP, local field potential

MD, mediodorsal nucleus of the thalamus

(m)PFC, (medial) prefrontal cortex

NMDA-R, N-methyl-D-aspartate receptors

norKet, (R,S)-norketamine

PCP, phencyclidine

RtN, reticular nucleus of the thalamus

SEM, standard error of means

V, ventral

Veh, vehicle

Abstract

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Key words: NMDA receptor antagonists; ketamine; thalamo-cortical networks; neuronal oscillations; single unit recordings; local field potentials.

Highlights:

- PCP and ketamine differentially affect thalamo-cortical activity anesthetized in rats
- As phencyclidine, ketamine inhibits GABA neurons of the reticular thalamic nucleus
- Unlike phencyclidine, ketamine does not excite MD/CM nuclei excitatory neurons
- Ketamine also inhibits layer VI pyramidal neurons in medial prefrontal cortex
- In parallel, ketamine enhances gamma band power and reduces delta band power

Introduction

Major depressive disorder (MDD) is a severe psychiatric disorder with a high prevalence and socio-economic impact worldwide (Kassebaum et al.; Kessler et al., 2003; Murray et al., 2012). Current monoaminergic antidepressants show slow onset of antidepressant action and limited efficacy (Penn and Tracy, 2012; Rush et al., 2006; Stahl, 2000; Trivedi et al., 2006). Therefore, there is an urgent need to develop faster and more effective treatments.

A substantial amount of evidence implicates the glutamatergic system in the pathophysiology and treatment of mood disorders (Beneyto et al., 2007; Feyissa et al., 2009; Hasler et al., 2007; Sanacora, 2009; Sanacora et al., 2012; Skolnick et al., 1996). Functional NMDA-R antagonists exhibit antidepressant-like effects in rodents (Skolnick et al., 2009), and, sub-anesthetic doses of the non-competitive NMDA-R antagonist ketamine has been shown to evoke immediate and persistent antidepressant responses in treatment-resistant depressed patients and antidepressant-like effects in rodents (Browne and Lucki, 2013; Mion and Villevieille, 2013; Monteggia and Zarate, 2015; Newport et al., 2015; Singh et al., 2016; Zarate et al., 2006). However, since ketamine treatment is limited by its psychotomimetic properties and abuse potential (Sanacora et al., 2017), there is a need to understand the mechanism(s) underlying its antidepressant action, ultimately leading to the development of more effective antidepressant drugs devoid of ketamine's side effects.

Non-competitive NMDA-R antagonists may preferentially block NMDA-Rs in GABAergic neurons thereby enhancing excitatory neurotransmission and glutamate release (Homayoun and Moghaddam, 2007; Jackson et al., 2004; Kargieman et al., 2007; Moghaddam et al., 1997). Ketamine's action appears to involve activation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPA-Rs) (Autry et al., 2011; Fukumoto et al., 2016; Koike and Chaki, 2014; Li et al., 2010; Maeng et al., 2008; Zanos et al., 2016), that subsequently may evoke biochemical/structural changes, increasing mTOR signaling and synaptogenesis (Duman and Aghajanian, 2012; Li et al., 2010; Popp et al., 2016). This might explain the long-lasting effects of ketamine that outlast the actual drug exposure (Mion and Villevieille, 2013).

The ventral anterior cingulate cortex in humans or its homologous region in rodents, the infralimbic (IL) subdivision of the medial prefrontal cortex (mPFC), is a sensitive area for the ketamine-mediated effects (Downey et al., 2016; Fuchikami et al., 2015; Li et al., 2016). Likewise, AMPA-R stimulation in IL evokes antidepressant-like effects in rats (Gasull-Camos et al., 2017). Given the reciprocal connectivity between the PFC and many subcortical brain areas (Gabbott et al., 2005; Groenewegen and Uylings, 2000), it is still unclear whether the actions of NMDA-R antagonists on PFC activity are locally or distally mediated.

Among the latter areas, several thalamic nuclei, in particular the mediodorsal nucleus (MD), make up the most important subcortical glutamate source to the PFC (Groenewegen and Uylings, 2000; Kuroda et al., 1998). In support of this, electrical stimulation of MD evokes AMPA-R- and NMDA-R-mediated responses in mPFC pyramidal neurons (Pirot et al., 1995). Interestingly, the selective chemogenetic activation of the MD inputs to the mPFC is sufficient to elicit antidepressant-like effects (Miller et al., 2017). Moreover, neuroimaging studies have shown that ketamine affects thalamic function (Downey et al., 2016; Maltbie et al., 2016; Vollenweider and Kometer, 2010). Collectively, these observations suggest an involvement of thalamo-cortical pathways in the action of ketamine, as previously observed for phencyclidine (PCP), another non-competitive NMDA-R antagonist. PCP has also been shown to elicit weak antidepressant-like effects in one rodent study (Hillhouse et al., 2014). In anesthetized rats, PCP inhibits GABAergic neurons from the reticular nucleus of the thalamus (RtN) (Troyano-Rodriguez et al., 2014), which tonically inhibits the rest of the thalamic nuclei. This effect results in the activation of a large percentage of thalamic neurons in the MD and centromedial (CM) nuclei and of mPFC pyramidal neurons (albeit a smaller percentage are inhibited by PCP), leading to increased c-fos expression in many cortical and thalamic areas (Kargieman et al., 2007; Santana et al., 2011).

In the present study we have investigated the actions of ketamine on thalamo-cortical networks using *in vivo* electrophysiology in anesthetized rats to increase knowledge of the brain circuitry affected by ketamine and to elucidate the mechanism(s) underlying its antidepressant effects. We have used the same experimental conditions and setting as previous electrophysiological studies from this laboratory on PCP (see above), which allows a direct comparison of the effects of both NMDA-R antagonists.

1. Methods and Materials

2.1. Animals. Animal care followed EU regulations (directive 2010/63 of 22 September 2010) and was approved by the Institutional Animal Care and Use Committee. Male albino Wistar rats (Charles River; 250-350 g) were kept under standard temperature (22 ± 1.5 °C) and humidity (55-65%) controlled laboratory conditions with food and water *ad libitum* in a 12 h: 12 h light/ dark cycle.

2.2. Drugs. (*R,S*)-Ketamine (Ketolar 50 mg/ml, Pfizer) was dissolved in 0.9% saline (0.5 mg/ml) and administered at cumulative doses of 0.25, 0.5, 1, 2 and 5 mg/kg, chosen based on the pharmacokinetic profile of ketamine to achieve brain exposures in rats comparable to the clinical concentrations (Shaffer et al., 2014). All drugs and vehicles (veh) were administered intravenously (i.v.) through a femoral vein cannula in chloral hydrate anesthetized rats. The following dosing paradigms were used (injection every 5 min): veh/veh1/veh2/veh3, veh/Ket0.25/Ket0.5 and veh/Ket1/Ket2/Ket5 (n=5-15 per group, Supplementary Table 1).

2.3. Surgical procedure. Rats were anesthetized with chloral hydrate (450 mg/kg, i.p. followed by 50-70 mg/kg per h i.p. using a perfusion pump and supplemented by 0.2 ml i.v. injection if required). A femoral vein cannula (P20 tubing) was inserted, and the rats were placed in a stereotaxic frame (David Kopf, Tujunga, CA) and kept at 37 °C via a heating pad (Cibertec RTC1, Madrid, Spain). One small hole was drilled into the skull to accommodate a glass electrode in either the RtN, the MD/CM or layer VI of the mPFC (coordinates relative to bregma (Paxinos and Watson, 2007) RTN: AP (anterior posterior): -1.4 to -3.14 mm, L (lateral): -1.8 to -3.8 mm, V (ventral): -5 to -6.5 mm; MD/CM: AP: -2.5 mm, L: -0.6 to -0.8 mm, V: -4.7 to -6.2 mm; mPFC: AP: +3.1 to +3.4 mm, L: -0.6 to -1 mm, V: -1.4 to -4.4 mm). Additionally, in the mPFC recordings, a metal electrode was inserted in the infralimbic cortex (IL) (AP: +3.2 mm, L: +0.7 mm, V: -4.2 mm) and a stimulating electrode was also placed in the MD (AP: -2.5 mm, L: -0.6 mm, V: -5.2 mm). A 2-mm screw was fixed in the skull to act as a reference/ground with a platinum wire.

2.4. Electrophysiological recordings. Single unit and local field potential (LFP) recordings in the RtN, MD/CM and layer VI of the mPFC were performed as previously described (Kargieman et al., 2007; Santana et al., 2011; Troyano-Rodriguez et al., 2014). Additional LFPs were recorded in the contralateral IL simultaneously with single unit and LFP recordings with glass electrodes using unipolar stainless steel

electrodes (single unit band-pass filter: 30 Hz-10 kHz; LFPs band-pass filter of 0.1–100 Hz in RtN and MD/CM and 0.1-200 Hz in mPFC).

All recorded units in the mPFC were identified as cortico-thalamic neurons by antidromic activation from the MD (electrical stimulation of the MD: square-wave pulses -0.5 ms duration, 250-500 μ A intensity- delivered at frequencies of 3-10 Hz) using a bipolar concentric tungsten electrode (TM53CCINS, World Precision Instruments Inc., USA) on basis of their fixed latency and collision with a spontaneous spike as described in a previous study (latency: 18.5 ± 3.4 ms, $n=29$) (Pirot et al., 1994). A scheme of the recording areas, their reciprocal connectivity and representative examples of single unit recordings is given in Figure 1.

2.5. Data analysis. To assess the acute effects of ketamine at different doses, each treatment was recorded for 5 min, of which the last 2 min periods were averaged and normalized to a stable 5 min baseline for the firing rate (Figures 2A, 3A, 4A) and to the last 1 min baseline for the oscillatory activity (Figures 2B, 3C, 4B). Similarly, to visualize prolonged effects of the higher dose administrated, the last 2 min period per 5 min bins were averaged and normalized to a stable 5 min baseline for the firing rate (Figure 3B) and to the last 1 min baseline for the oscillatory activity (Figures 3D-E, 4C-D). Analysis of single unit recordings was carried out importing the signals to MATLAB (MathWorks, Natick, Massachusetts) for off-line analysis. A burst episode was defined as the occurrence of two or more spikes with an interspike interval of ≤ 4 ms for the MD, ≤ 10 ms for the RtN and < 45 ms for the mPFC (Kargieman et al., 2007; Laviolette et al., 2005; Santana et al., 2011; Sittig and Davidowa, 2001; Troyano-Rodriguez et al., 2014; Ushimaru et al., 2012). LFP recordings were Fast Fourier transformed to yield the power of oscillatory activity of 1 min signal intervals (Spike 2 software version 7.10, Cambridge Electronic Design Limited, UK). LFP signals were divided into 5 main frequency bands of oscillations: delta 0.15-4, theta 4-8 Hz, alpha 8-12 Hz, beta 12-30 Hz, gamma 30-90 Hz or low gamma 30-48 Hz. Data from 48 to 52 Hz and 58 to 62 Hz frequency range was discarded due to electric noise. One way repeated measures analysis of variance (RM-ANOVA) as between-subject factor and treatment or time as within-subject factor followed by Holm-Sidak post-hoc test were used to assess the differences between baseline and post-injection values of firing rate and power of the oscillations within the same treatment group (Sigma Plot, version 11.0). Data are presented as mean $\pm$ standard error of the means (S.E.M.) with a p value of < 0.05 being considered significant.

2.6 Tissue and plasma collection. Plasma and brain concentrations of (*R,S*)-ketamine, (*R,S*)-norketamine and (*2R,6R*)-hydroxynorketamine (HNK) were measured in some animals at the end of the recordings (indicated in Supplementary Figure 4), either 7 min or 45 min after the last compound administration. At the terminal time-point, a blood sample was taken by heart puncture and kept in 1.6 mg EDTA/mL of blood. Brains were stored at -80 °C until they were sectioned at 40 µm in serial coronal slices that were stained with neutral red to inspect the electrode placement. Blood samples were centrifuged for 10 min at 5000 g at 4 °C and plasma was stored at -80 °C until analysis.

2.7. Quantitative bioanalysis. Rat plasma and brain concentrations of ketamine, norketamine and HNK were determined using ultra performance liquid chromatography coupled to tandem mass spectrometry (MS/MS). Plasma and brain concentrations of the compounds were analyzed by unpaired student t-test using SigmaPlot (version 11.0).

3. Results

3.1 Firing patterns of reticular, thalamic and cortical neurons.

The mean firing rates of the recorded neurons during 5 min baseline were: 11.9 ± 2.3 spikes/s in the RtN ($n=18$), 1.5 ± 0.3 spikes/s in the MD/CM ($n=23$) and 1.5 ± 0.4 in the mPFC ($n=29$). In the RtN, most neurons discharged in burst firing mode. In the MD/CM, both depolarized and hyperpolarized action potentials were found. Burst episodes were characterized by doublets and triplets. Antidromically-driven cortico-thalamic pyramidal neurons exhibited a broad pattern of spontaneous activity but most units exhibited irregularly spaced bursts. These firing characteristics are consistent with those observed in previous studies (Pirot et al., 1994; Santana et al., 2011; Troyano-Rodriguez et al., 2014). Representative examples of the firing patterns are given in Figure 1 together with a schematic representation of the projections in the thalamo-cortical network. Systemic administration of vehicle did not significantly affect the firing rate or oscillatory activity in the RtN, MD/CM or mPFC (Supplementary Figure 1).

3.2. Effects of ketamine on the activity of RtN.

The effect of ketamine on the discharge of RtN neurons was studied in 13 rats at cumulative doses of 1, 2 and 5 mg/kg (one neuron per rat). One more rat was added for the analysis of the oscillatory activity ($n=14$). Ketamine significantly reduced the firing activity of RtN neurons to 61%, 65% and 37% of baseline at 1, 2 and 5 mg/kg, respectively ($F_{4,47}=13.16$, $p<0.001$, $n=12-13$) (Figure 2A). Ketamine also significantly decreased the number of burst episodes but did not alter the burst pattern of these neurons (Supplementary Table 2A).

In parallel with the effects on neuronal discharge, ketamine dose-dependently decreased delta (0.15-4 Hz) power to 79%, 71% and 48% of baseline, at 1, 2 and 5 mg/kg, respectively ($F_{4,52}=23.46$, $p<0.001$, $n=14$), whereas the power of other frequency bands remained unaltered (Figures 2B, 2C).

3.3. Effects of ketamine on the activity of MD/CM.

The effect of ketamine on MD/CM activity was studied in 5 rats at 0.25 and 0.5 mg/kg (cumulative doses) and in 12 rats at 1, 2 and 5 mg/kg (cumulative doses; one neuron per rat). Analysis of the oscillatory activity was carried out in the same 12 rats and in one additional rat ($n=13$). In some rats ($n=6$), recordings were maintained up to 30 min after injection of the last dose in order to explore the potentially prolonged effects of the drug.

Ketamine did not significantly affect MD/CM activity at 0.25 and 0.5 mg/kg ($n=5$) (Supplementary Figures 2, A1-A2). However, ketamine dose-dependently reduced the firing activity of MD/CM neurons to 66%, 60% and 37% of the baseline at 1, 2 and 5 mg/kg, respectively ($F_{4,42}=22.79$, $p<0.001$, $n=11-12$) (Figure 3A). In the units examined for 30 min ($n=6$), the firing rate returned to baseline approximately 15 min after administration, consistent with the rapid pharmacokinetics of the drug ($F[\text{treatment}]_{4,20}=15.84$, $p<0.001$, $n=6$) (Figure 3B). Ketamine (1, 2, 5 mg/kg, i.v.) significantly decreased the number of burst episodes, the number of spikes per burst and the burst duration (Supplementary Table 2B).

In parallel, ketamine significantly increased low gamma (30-48 Hz) power to 137%, 140% and 138% of the baseline at 1, 2 and 5 mg/kg, respectively ($F_{4,47}=17.30$, $p<0.001$, $n=13$) (Figure 3C). This effect was sustained for up to 15 min post-administration of the last dose ($F_{7,34}=3.70$, $p<0.01$, $n=6$) (Figures 3D, 3E). In contrast, ketamine (5 mg/kg, i.v.) evoked a significant and transient decrease of delta power to 73% of the baseline ($F_{4,47}=5.70$, $p<0.001$, $n=13$) (Figures 3C-3E).

3.4 Effects of ketamine on the activity of layer VI pyramidal neurons in mPFC

The effect of ketamine on the discharge of cortico-thalamic neurons was studied in 5 rats at the dose of 0.25 mg/kg and in 15 rats at the cumulative doses of 1, 2 and 5 mg/kg (one neuron per rat). Oscillatory activity was additionally recorded with a metal electrode placed in the layer VI of the infralimbic cortex in the contralateral hemisphere. Analysis of the oscillatory activity in the 0.25 mg/kg group was done in the same 5 rats, and the effect was explored up to 40 min post-administration. In the 1, 2 and 5 mg/kg group, the analysis was carried out in 14 rats, and in 12 of them, the recordings could be maintained for up to 30 min after the last drug administration in order to explore prolonged effects of ketamine.

As in MD/CM, the low ketamine dose (0.25 mg/kg, i.v.) did not significantly affect layer VI mPFC activity ($n=5$) (Supplementary Figures 2 B1 and B2), while it dose-dependently reduced the firing rate to 74%, 53% and 19% of the baseline at 1, 2 and 5 mg/kg, respectively ($F[\text{treatment}]_{4,53}=8.10$, $p<0.001$, $n=13-15$) (Figure 4A). As in MD/CM, the firing rate returned to baseline approximately 15 min after administration (Supplementary Table 2C). Ketamine also decreased the number of burst episodes but did not alter the burst pattern of the recorded neurons (Supplementary Table 2C).

Ketamine evoked a transient and significant decrease of delta power (0.15-4 Hz) to 89%, 85% and 68% at 1, 2 and 5 mg/kg, respectively ($F_{4,52}[\text{treatment}]=7.70$, $p<0.001$, $n=14$) (Figure 4B). Figures 4C-4D show the temporal course of ketamine's effect ($F_{7,77}[\text{treatment}]=5.18$, $p<0.001$, $n=12$).

In parallel, ketamine (1, 2 and 5 mg/kg, i.v.) significantly increased beta and gamma powers (12-30 Hz; 30-90Hz, respectively) to 123%, 129%, 120% and 135%, 137%, 115% of the baseline, respectively (beta: $F[\text{treatment}]_{4,52}=6.21$, $p<0.001$; gamma: $F[\text{treatment}]_{4,52}=7.46$, $p<0.001$, $n=14$) (Figure 4B). Unlike for delta power, ketamine evoked a prolonged increase in beta and gamma powers that persisted for up to 30 min post-administration (beta: $F_{7,77}[\text{treatment}]=8.07$, $p<0.001$; gamma $F_{7,77}[\text{treatment}]=9.92$, $n=12$) (Figures 4C, 4D). Similar changes on oscillatory activity were observed with glass electrode recordings (Supplementary Figure 3).

3.5 Terminal plasma and brain concentrations of ketamine and its metabolites

Plasma and brain concentrations of ketamine and its major metabolites, norketamine and HNK (Desta et al., 2012) were determined in a subgroup of animals at the end of the recordings. The concentrations of ketamine, norketamine and HNK in plasma and brain 7 and 45 min after the last cumulative dose of 5 mg/kg i.v. are given in Supplementary Figure 4. Ketamine concentrations in plasma and brain at 45 min were lower than at 7 min post-administration ($p<0.01$, unpaired student's t-test) (Supplementary Figure 4). In contrast, HNK concentrations in plasma and brain at 45 min post-administration were significantly higher than at 7 min post-administration ($p<0.01$, unpaired student's t-test). Norketamine concentrations in plasma and brain were not significantly different at the two different sampling times (n.s., unpaired student's t-test) (Supplementary Figure 4). In brain, the main components at 7 min post-administration were ketamine and HNK (mean ketamine/HNK ratio: 2.9) whereas at 45 min, both compounds were found at comparable concentrations (mean ketamine/HNK ratio: 0.9) (Supplementary Figure 4B).

4. Discussion

In the present study we show that ketamine inhibits the firing activity of thalamo-cortical networks in anesthetized rats. Ketamine dose-dependently decreased the discharge of both excitatory MD/CM relay neurons projecting to PFC as well as layer VI cortico-thalamic pyramidal neurons, despite that ketamine markedly suppressed the discharge of RtN GABAergic neurons known to exert tonic inhibition of the rest of the thalamus. In parallel, ketamine affected population activity in these areas, thus evoking a short-lasting decrease of low frequency oscillations and a more prolonged increase of gamma oscillations in MD/CM and PFC. Except in the RtN, the effects of ketamine were strikingly opposite of those evoked by PCP, which activates thalamo-cortical networks (Kargieman et al., 2007; Santana et al., 2011; Troyano-Rodriguez et al., 2014). The data from these PCP studies and the present ketamine study are directly comparable since they were conducted with the same experimental conditions in the same setting (see Supplementary Table 3). Likewise, we used doses of both compounds which were comparable in terms of NMDA-R antagonism (Anis, 1983). Importantly, the brain concentrations determined for ketamine in this study were within the range reported in clinical and preclinical studies reporting antidepressant effects (Li et al., 2011; Shaffer et al., 2014), supporting the relevance of these findings for the antidepressant action of ketamine.

4.1 Acute effect of ketamine on the activity of thalamo-cortical networks.

As observed with PCP (Troyano-Rodriguez et al., 2014) and in agreement with the hyperpolarization of RtN neurons mediated by the NMDA-R antagonist APV *in vitro* (Zhang et al., 2009), ketamine markedly inhibited the activity of GABAergic RtN neurons. However, in contrast to PCP (Santana et al., 2011), this effect did not translate into an increased activity of MD/CM neurons. Instead, ketamine acutely attenuated the discharge of excitatory MD/CM neurons that mainly project to layers III and V-VI of the PFC (Kuroda et al., 1998) and of cortico-thalamic neurons in layer VI of mPFC. Similarly, MK-801 evoked an overall decrease of spiking activity in the mPFC of urethane-anesthetized rats (Kiss et al., 2011)

Most cortical pyramidal and thalamic relay neurons express NMDA-Rs (Monyer et al., 1994; Wenzel et al., 1997). Therefore, the reduction in firing rate induced by ketamine in these areas is likely to result from a direct blockade of NMDA-Rs in the recorded neurons. In contrast, previous observations in PFC and MD/CM indicate that PCP in anesthetized and MK-801 in awake rats, preferentially increase excitatory neuron discharge after the blockade of NMDA-Rs on local or distal GABAergic elements (Homayoun and Moghaddam, 2007; Jackson et al., 2004; Jodo et al., 2003; Kargieman et al., 2007; Santana et al., 2011; Troyano-Rodriguez et al., 2014). In the latter studies, inhibitory actions of PCP were

actually also observed in mPFC and MD/CM, yet in a lower percentage of neurons than that of excited neurons, thus resulting in an overall increase of discharge (Supplementary Table 3). These differences suggest that the net effect of any given NMDA-R antagonist on thalamo-cortical neurons is determined by the balance between the inhibitory effect induced by its NMDA-R blockade in the recorded unit and the disinhibition evoked by NMDA-Rs located on GABAergic elements controlling thalamic and cortical activity (e.g., GABA interneurons in PFC, RtN and basal ganglia structures controlling the activity of excitatory thalamic nuclei). Given that ketamine and PCP inhibit RtN neurons to a similar extent, their differential effect in MD/CM and mPFC is possibly due to a more potent and simultaneous blockade of NMDA-Rs by ketamine in MD/CM and PFC, which occludes the disinhibition produced by the reduction of RtN activity (see scheme in Figure 5).

Several mechanisms could underlie this difference between PCP and ketamine. Although ketamine, MK-801 and PCP essentially are non-selective antagonists of the different GluN1/2 NMDA-R subtypes (Dravid et al., 2007; Yamakura et al., 1993), ketamine exhibits some preference for recombinant GluN2C- and 2D-containing receptors over the other GluN1/2 subtypes in presence of Mg^{2+} (Khlestova et al., 2016; Kotermanski and Johnson, 2009). Given the preferential expression of the GluN2C subunits in thalamus (Karavanova et al., 2007; Monyer et al., 1994; Wenzel et al., 1997) this might partly explain a preferential NMDA-R blockade by ketamine in the excitatory thalamic nuclei, thus counteracting the disinhibition induced by NMDA-R blockade in RtN and the subsequent reduction of GABA inputs onto MD/CM neurons. The actions of both ketamine and PCP are voltage-dependent, with ketamine exhibiting a higher voltage-dependency than PCP (Davies et al., 1988; Gilling et al., 2009; MacDonald et al., 1991). This suggests that PCP and ketamine may exert differential actions on the recorded neurons, depending on their depolarization state. Likewise, despite both agents were used at subanesthetic doses, a differential effect of PCP and ketamine on chloral hydrate-induced anesthesia may also contribute. Indeed, anesthesia is known to impact on the excitatory/inhibitory balance of these neurons. Hence, RtN neurons are more hyperpolarized under anesthesia (Pinault, 2004; Yen and Shaw, 2003) while they are depolarized in awake animals. Indeed, the main limitation of this one and previous studies is the use of anesthesia, which warrants further investigation of the effects of these two agents on neuronal activity in freely-moving rats.

In agreement with the present observations, ketamine has previously been reported to reduce metabolic activity in thalamic nuclei of mice, including RtN and MD, in parallel with an increased activity of upper cortical layers (I-III) of the mPFC (Dawson et al., 2014; Dawson et al., 2013). However neuroimaging studies in healthy volunteers and depressed patients as well as in non-human primates

have reported that ketamine increases glucose metabolism in the cingulate cortex and the thalamus (De Simoni et al., 2013; Downey et al., 2016; Doyle et al., 2013; Maltbie et al., 2016; Vollenweider and Kometer, 2010). These discrepancies are not fully understood and may involve several factors, including *i)* species differences, perhaps related to the presence of thalamic interneurons in primate and human brains, but not in rodent brain, except for the lateral geniculate nucleus (Arcelli et al., 1997; Ohara et al., 1983), *ii)* differential target selectivity of ketamine at the very different doses and routes used in these studies, *iii)* variable concentrations of ketamine and metabolites at the different time points reported in the above studies [This point may be particularly relevant, given the putative involvement of (2S,6S;2R,6R)-HNK metabolite in the antidepressant effects of ketamine (Zanos et al., 2016)], *iv)* the use of anesthesia in some studies, since ketamine increases glucose metabolism in awake but not in anesthetized rodents, and most preclinical fMRI studies are conducted in anesthetized animals (Littlewood et al., 2006), and *v)* differential effects depending on the administration route and rate of infusion.

Together with the decrease in neuronal discharge, ketamine transiently decreased delta (0.15-4 Hz) power in the RTN, MD/CM and mPFC (IL cortex). Similarly, PCP acutely decreased delta oscillations in anesthetized rats (Kargieman et al., 2007; Neal and Bond, 1985; Santana et al., 2011; Troyano-Rodriguez et al., 2014). Delta oscillations reflect synchronized activity changes of neuronal networks and are generated by neocortical and thalamo-cortical networks (Steriade et al., 1993; Timofeev and Chauvette, 2011). Interestingly, the acute reductions in delta power mediated by ketamine are short-lasting, reflecting its fast pharmacokinetics. The association of this effect to its psychotomimetic activity is supported by the short-lasting perceptual disturbances induced by i.v. ketamine in clinical studies (Zarate et al., 2006). Likewise, the reduction in delta power induced by PCP following its i.v. administration is reversed by marketed and experimental antipsychotic drugs (Kargieman et al., 2007; Llado-Pelfort et al., 2016; Santana et al., 2011; Troyano-Rodriguez et al., 2014).

4.2 Prolonged effect of ketamine on thalamo-cortical activity

The increase in gamma oscillations in mPFC and MD/CM nuclei mediated by ketamine were characterized by a distinct temporal pattern compared to its reduction of delta oscillations as this increase was observed throughout the recording period and long after delta oscillations had returned to baseline values. Given the likely association of the latter parameter to ketamine's psychotomimetic effects, the persisting increase in gamma power may possibly be associated with ketamine's antidepressant effects as these occur after the psychotomimetic phase of the drug.

NMDA-R antagonists also increase gamma power in the cortex of anesthetized and freely moving animals (Hakami et al., 2009; Hiyoshi et al., 2014; Kittelberger et al., 2012; Phillips et al., 2012; Pinault, 2008; Sanacora et al., 2014; Wood et al., 2012; Zanos et al., 2016), which suggests that the anesthetized preparation is predictive of effects in freely-moving rodents, without the potential confounding effects of behavioral activity in awake animals. Gamma oscillations reflect local cell assembly organization in cortical networks. They depend on the activation of ionotropic excitatory receptors and are therefore considered to reflect neuronal excitation/disinhibition (Buzsaki and Draguhn, 2004; Cunningham et al., 2003; Whittington et al., 2000). Therefore, the ketamine-induced increase of gamma oscillations in mPFC may be taken as a surrogate measure of an increase in PFC (in particular, IL) excitability. Since the present single unit data enables to discard an increased excitatory thalamic input as a potential source of this effect, this suggests an involvement of other cortical or subcortical excitatory inputs. Among the former inputs, blockade of NMDA-Rs in fast-spiking GABAergic interneurons may be involved in gamma generation in cortical networks (Cardin et al., 2009; Carlen et al., 2012) and excitation of cortical pyramidal neurons (Hanson et al., 2013; Homayoun and Moghaddam, 2007; Jackson et al., 2004; Quirk et al., 2009). However, both effects are unlikely to occur in layer VI, at least in the short run, given the inhibitory effect of ketamine in cortico-thalamic pyramidal neurons. However, by virtue of the differential connectivity of pyramidal neurons in middle and deep mPFC layers in the various subfields (Gabbott et al., 2005), we cannot rule out a ketamine action on other PFC layers. A further in-depth analysis of this effect is certainly warranted, given its potential association with ketamine's antidepressant effects.

The temporal dissociation between the effect of ketamine on delta and gamma oscillations (with more persistent effects on the latter) is reminiscent of the i.v. effects of ketamine in patients, with a persistent antidepressant effect by the end of the psychotomimetic phase (Zarate et al., 2006) that follows the rapid pharmacokinetics of ketamine (Mion and Villevieille, 2013). HNK has been suggested to mediate the delayed antidepressant effect through the activation of AMPA-Rs (Zanos et al., 2016) although a very recent study indicates that HNK also blocks synaptic NMDA-R function, as observed for the parent compound (Suzuki et al., 2017). Interestingly, the present data show that the increase in gamma power occurs after a switch in the relative concentration of ketamine and its metabolites with the HNK/ketamine ratio in brain being higher 45 min after than that 7 min after administration. This observation is consistent with Zanos et al., 2016 (Zanos et al., 2016), showing that HNK administration may increase gamma power in mouse cortex. Overall, these findings suggest a temporal association between the prolonged increase in gamma power and the antidepressant-like effects of ketamine,

inasmuch as the NMDA-R-antagonist-induced gamma oscillations could not be prevented by antipsychotic drugs (Jones et al., 2012). However, further investigations are needed to validate this variable as a potential biomarker.

In summary, the present study indicates that ketamine evokes a prominent fast and short-lasting decrease of the activity of thalamo-cortical circuits together with a decrease in delta oscillations, followed by a persistent increase of gamma oscillations. Further work is needed to address the functional implications of these observations and to fully understand the putative contributions of these effects to the psychotomimetic and antidepressant effects of ketamine. Finally, the direct comparison of the present ketamine study and past PCP studies (Kargieman et al., 2007; Santana et al., 2011; Troyano-Rodriguez et al., 2014) allows us to conclude that non-competitive NMDA-R antagonists have markedly different, sometimes opposite, actions on neuronal activity, and cannot be taken as a homogenous group in regards to their *in vivo* actions on brain function.

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Conflict of interest

FA has also received consultation fees from Lundbeck A/S and is scientific advisor to Neurolix. FA and PC are PI and co-PI of a research contract with Lundbeck A/S. MAF, KFH and NP are Lundbeck A/S employees. AAJ reported no potential conflicts of interest.

Figure Legends

Figure 1. Recording sites and firing characteristics. **(A)** Schematic representation of thalamo-cortical projections. **(B)** Representative examples of firing characteristics of mediodorsal and centromedial thalamic (MD/CM) neurons, reticular thalamic (RtN) neurons and cortico-thalamic neurons in layer VI of the medial prefrontal cortex (mPFC). **(C)** Histological verification of representative neurons in the three different recording sites.

Figure 2. Effects of ketamine (Ket; 1, 2, 5 mg/kg i.v.) treatment on firing rate **(A)** and oscillatory activity **(B and C)** of RtN neurons. **(A)** Bar histogram representing the firing rate of the averaged last 2 min periods from a 5 min recording per treatment. The percentage represents the 2 min bins normalized to a stable 5 min baseline (n=13-12) (* p<0.05 vs. Basal, # p<0.05 vs. vehicle (Veh), + p<0.05 vs. Ket 2 mg/kg). **(B)** Bar histogram representing the mean power (last 2 min periods from a 5 min recording per treatment) of each frequency band relative to the last 1 min baseline (n=14) (* p<0.05 vs. Veh, # p<0.05 vs. Ket 1 mg/kg). **(C)** Color-coded spectrogram plotted with 1 Hz resolution (y-axis). Each horizontal time bin represents the average spectral power per 1 min time bin normalized to the 5 min baseline period before vehicle administration (n=14). Data are presented as average or percentage (%) ± SEM.

Figure 3. Effects of ketamine (Ket; 1, 2, 5 mg/kg i.v.) treatment on firing rate **(A and B)** and oscillatory activity **(C, D and E)** of MD/CM neurons. **(A)** Bar histogram representing the firing rate of the averaged last 2 min periods from a 5 min recording per treatment. The percentage represents the 2 min bins normalized to a stable 5 min baseline (n=12-11) (* p<0.05 vs. Basal, # p<0.05 vs. vehicle (Veh), α p<0.05 vs. Ket1, + p<0.05 vs. Ket2). **(B)** Bar histogram representing the effect of ketamine 5 mg/kg i.v. (cumulative dose) on the firing rate up to 15 min post-administration (n=6) (* p<0.05 vs. Basal, # p<0.05 vs. Veh, ^ p<0.05 vs. Ket 5 at 15 min). **(C)** Bar histogram representing the mean power (last 2 min periods from a 5 min recording per treatment) of each frequency band relative to the last 1 min baseline (n=13) (* p<0.05 vs. veh, # p<0.05 vs. ket1). **(D)** Line histogram representing the mean power (last 2 min periods of 5 min bins) of each frequency band relative to the last 1 min baseline (n=6) (* p<0.05 vs. min 10, # p<0.05 vs. min 25). **(E)** Color-coded spectrogram plotted with 1 Hz resolution (y-axis). Each horizontal time bin represents the average spectral power per 1 min time bin normalized to the 5 min baseline period before vehicle administration (n=6). Data are presented as average or percentage (%) ± SEM.

Figure 4. Effects of ketamine (Ket; 1, 2, 5 mg/kg i.v.) treatment on firing rate of antidromically driven cortico-thalamic neurons (A) and oscillatory activity of layer VI infralimbic (IL) cortex (B, C and D). **(A)** Bar histogram representing the firing rate of the averaged last 2 min periods from a 5 min recording per treatment. The percentage represents the 2 min bins normalized to a stable 5 min baseline (n=15-13) (* $p < 0.05$ vs. Basal, # $p < 0.05$ vs. Veh $\times$ $p < 0.05$ vs. Ket 1 mg/kg). **(B)** Bar histogram representing the mean power (last 2 min periods from a 5 min recording per treatment) of each frequency band relative to the last 1 min baseline (n=14) (* $p < 0.05$ vs. veh). **(C)** Line histogram representing the mean power (last 2 min periods from a 5 min recording per treatment) of each frequency band relative to the last 1 min baseline (n=12) (* $p < 0.05$ vs. min 10, # $p < 0.05$ vs. min 25). **(D)** Color-coded spectrogram plotted with 1 Hz resolution (y-axis). Each horizontal time bin represents the average spectral power per 1 min time bin normalized to the 5 min baseline period before vehicle administration (n=12). Data are presented as average or percentage (%) $\pm$ SEM.

Figure 5. Schematic representation of thalamo-cortical connectivity (modified after Tronyano-Rodriguez et al., 2014) (Troyano-Rodriguez et al., 2014) with representative examples of the effect of ketamine on neuronal discharge in each of the areas examined. Ketamine induced a dramatic reduction of the discharge of GABAergic RtN neurons that tonically inhibit excitatory thalamic nuclei. Despite the reduced GABAergic inputs onto MD/CM neurons resulting from ketamine's effect on RtN neurons, ketamine reduced the neuronal discharge in these thalamic nuclei, as well as in layer VI pyramidal neurons of the mPFC which project to thalamic excitatory (MD/CM among others) and inhibitory (RtN) nuclei. The activity of excitatory thalamic nuclei is also controlled by GABAergic structures of the basal ganglia circuit, such as ventral pallidum and substantia nigra reticulata (not examined in the present study).

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