

# Acute & sub-chronic neuronal effects of NMDA receptor antagonist, memantine using pharmacological magnetic resonance imaging

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**INTRODUCTION:** Despite of larger number of clinical observations it is still unknown and therefore subject to interpretation whether and how NMDA (N-methyl-D-aspartate) glutamate receptors are exactly involved in the pathogenesis of the neurodevelopment disorder, schizophrenia. An efficient tool for addressing this question is the use of compounds acting as non-competitive antagonists of the NMDA receptor including ketamine & phencyclidin. The use of these compounds changes the behavior in both healthy volunteers and animals, inducing psychomimetic syndrome which resembles schizophrenia (review: [1]) and while administered to schizophrenic patients the schizophrenic symptoms are exacerbated [2, 3]. The present study reports on the acute and sub-chronic neuronal effects of the NMDA antagonist memantine on the rat brain measured as BOLD (blood oxygenation level dependent) contrast changes in a pharmacological magnetic resonance imaging (phMRI) study. Corroborative investigations include recording the spontaneous local field potential (LFP) activity in key brain regions (through electrophysiology) and the pharmacokinetics of acute and sub-chronic memantine treatment in blood plasma and the brain.

**EXPERIMENTAL PROTOCOL & DATA ANALYSIS:** Six experimental groups of male Lister Hooded rats (250 to 280g; 6 per treatment group) were used. Acute treatment: while in-magnet, subjects received memantine (20 or 40mg/kg, IP) or saline IP. Sub-chronic treatment groups received daily pre-treatment with memantine (20 or 40 mg/kg, IP) or saline (IP) for 5 days, before the day of scanning; while in-magnet they had an acute IP injection, of the same study compound. Subjects were scanned in a 9.4T MRI (Bruker) scanner (Gradient Echo (GE); TR: 1048.32ms; TE: 17ms; FOV: 25x25mm<sup>2</sup>; Matrix size: 64x64; Slice thickness; 0.5mm; 32 slices). 120 function scans (~2 hrs) were acquired (30 baseline scans, followed by drug injection & continuous scanning). Images were registered, vascular masked (to suppress signal changes associated with macroscopic vessels [4]), and Gaussian smoothed. Parametric maps of statistical significance were obtained using fixed effects GLM, using SPM99. The condition tested was a comparison between pre & post test injection. Global muscle signal intensity time traces were determined & were used as covariates [5]. For electrophysiology: the rats from sub-chronic memantine (20mg/kg, IP) & saline treatment groups were further utilized. Under standard experimental conditions, silicon-based multielectrode arrays were implanted stereotactically in the rat prelimbic cortex and employed to record spontaneous local field potential activity (LFP). For pharmacokinetics: at time points of 1, 2, 4, 7, 24 hours post-injection (memantine 20mg/kg, IP) the exposures in plasma and brain were determined in separate groups (3 per treatment group) following acute and sub-chronic dose. Samples were analyzed using a research liquid chromatography-mass spectrometry (LC-MS/MS) method.

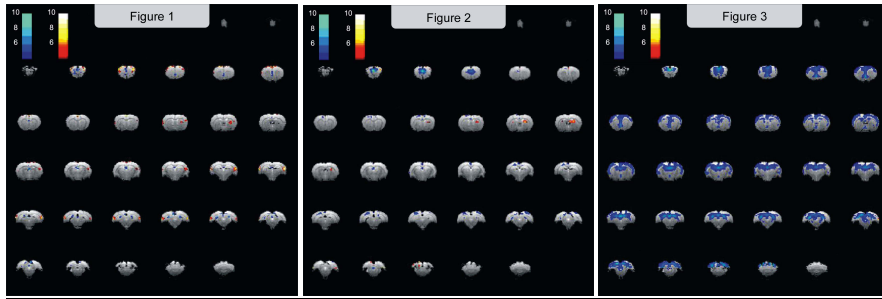
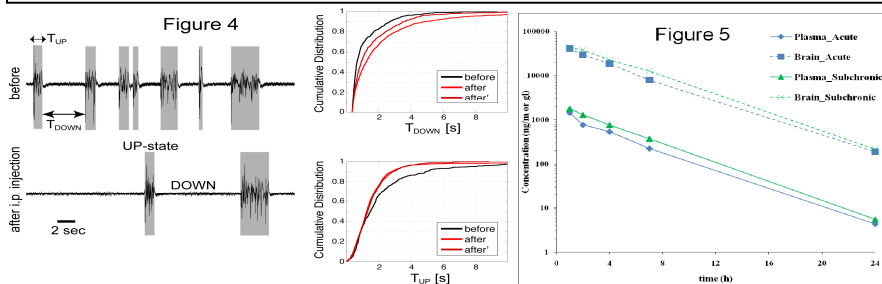


Figure 1-3: SPM {t} distribution maps representing BOLD signal changes overlaid on anatomical template image. Colored pixels represents significant correlation (threshold at  $p < 0.05$  corrected for multiple comparisons) of signal time course with input test-condition (pre vs. post test injection) in comparison to vehicle treated controls. Red and blue represents positive and negative correlations respectively. The 32 brain slices are contiguous, covering the entire brain.



**RESULTS:** The BOLD phMRI following acute memantine administrations produced highly significant dose dependent activation changes in the pre-limbic cortex; [Fig 1]: 20mg/kg IP and [Fig 2]: 40 mg/kg, IP memantine resp. Sub-chronic treatment with memantine (20 mg/kg, IP) produced significantly localized changes in pre-limbic & retrosplenial cortex and hippocampus (HC) [Fig 3]. With higher sub-chronic dose of 40mg/kg, IP memantine treatment adverse systemic effects were monitored. The nature of adaptations demonstrates the specific involvement of the key regions (pre-limbic cortex and hippocampus) integral with the drug action.

In **electrophysiology recordings**, the electrical activity of the prelimbic cortex was dominated by slow-wave synchronized activity, resulting into relatively variable epochs of bistable local electrical field-potential (LFP) (i.e. UP/DOWN states – Fig 4, left), coexisting with periods of relatively more desynchronized activity. As a result of memantine injection [Fig 4, right], the duration of the DOWN-states significantly increased ( $p < 10^{-18}$ , K-S test) while the duration of the UP-states significantly decreased ( $p < 10^{-5}$ ).

**Pharmacokinetics of memantine** (20mg/kg, IP) in plasma and brain revealed similar exposures (with comparable brain/plasma ratio) between acute and sub-chronic treatment [Fig 5].

**CONCLUSIONS:** The main finding to emerge from this study is the identification of the key regions implicated in the mechanism of action of NMDA receptor antagonist, memantine in inducing schizophrenia-like manifestations. Well integral with our regional responses, in a recent review Kristiansen et al., 2007 summarized the abnormalities of NMDA receptors in schizophrenia, in cingulate cortex and hippocampus in postmortem brain samples of humans. Similar regional specific phMRI responses in the frontal, hippocampal and limbic areas have also been previously reported in rats with an alternate NMDA antagonist – ketamine [7, 8]. Results from our electrophysiology observations indicate that the acute effect of IP memantine injection in sub-chronically treated animals consists in a reduction of intrinsic cortical activity in the prelimbic cortex, quantified in terms of number and duration of the UP-states. Although the conclusive link between brain hemodynamics & neuronal (electro)physiology is still under debate, it has been proposed that the BOLD response primarily reflects the input & local processing of neuronal information rather than the output signals [9]. Similarly, LFPs reflect primarily a weighted average of synchronized dendro-somatic components of the synaptic signals of a neural population, within 1-2 mm from the electrode tips. Thus it's conservable that the reduced synaptic electrophysiological response, directly correlate with the decreased BOLD response. All in all, since memantine antagonizes NMDAR-mediated glutamatergic activity, an overall decrease in recurrent synaptic coupling between cortical neurons is sufficient to account for the observed decrease in UP-states frequency and duration [10]. This supports that LFPs can be used to predict in part the BOLD signal [9]. Moreover observations from the exposures of plasma and brain following acute & sub-chronic treatment being comparable, validates the phMRI BOLD responses being reported (i.e. effects are not a resultant of drug accumulation). Our observations are of potential importance in understanding the role of NMDA receptor hypofunction in psychosis, which will find potential utility for further investigations involving antipsychotic drug testing's &/or studies on etiopathogenesis of schizophrenia.

**REFERENCES:** [1] Coyle et al., Ann. N.Y. Acad. Sci. 1003: 318-327, 2004; [2] Lahti et al., Neuropsychopharm 13: 9-19, 1995; [3] Bubenikova-Valesova V, et al., Neurosci & Beh Rev 32: 1014-1023, 2008; [4] Hlustik et al., Neuroimage 7(3): 224-231, 1998; [5] Lowe AS, et al., NMR in Biomed. 21: 53-58, 2008; [6] Kristiansen et al., Neurosci. 7: 48-55, 2007; [7] Littlewood CL, et al., Psychopharm. 186: 64-81, 2006; [8] Littlewood CL, et al., Neuroimage 32: 1733-1746, 2006; [9] Logothetis NK & Wandell BA, Annu Rev Physiol. 66: 735-769, 2004; [10] Giugliano M, et al., J. Neurophysiol 92(2): 977-996, 2004.