**Background**

N-methyl-D-aspartate receptor (NMDA-R) have been described as potential novel treatment options in the therapy of treatment-resistant depression (TRD). A single sub-anesthetic dose of the noncompetitive NMDA-R antagonist ketamine has been shown to exert rapid-onset and long-lasting antidepressant effects in TRD patients; these effects were accompanied by brief psychotic episodes. Fast-onset antidepressant effects without psychotic side effects have also been described in the clinic for the NR2B-selective negative allosteric modulator (NAM) traxoprodil. However, other unspecific NMDA-R blockers, such as lanicemine, failed to show fast-onset long-lasting antidepressant effects in the clinic after single application. Here, we used glutamate voltammetry in rats in order to identify a preclinical predictor of clinical efficacy by circuit engagement in the prefrontal cortex (PFC).

**Hypothesized antidepressant MoA of NMDA-R antagonists:**

Disinhibition of glutamatergic neurons in the PFC

**Advantages of glutamate voltammetry**

- fast response time
- fast sampling time (1 Hz)
- high sensitivity
- low analyte consumption
- no sample post-processing

**Experimental design**

Experiments were performed in Wistar Han rats implanted with guide cannulae at the specified coordinates. On the day prior to the experiment, sensors were implanted via the guide cannulae and allowed to equilibrate in vivo over night. Recordings were performed in the animals’ home cages. A baseline was recorded for at least 30 min and the animals were subsequently treated by intraperitoneal injection (i.p.) with the compounds or with vehicle. Treatment-elicted changes in glutamate levels were recorded for at least 1 h post-treatment.

**Glutamate voltammetry shows glutamate increase to be the efficacy-associated MoA for NMDA-R antagonists**

To investigate the relationship between circuit engagement and clinical efficacy, S-ketamine and traxoprodil, which have shown clinical efficacy, were compared with lanicemine, which failed in the clinic. Doses were selected by PK/PD modeling to mirror 0.3fold, 1fold and 3fold clinically efficacious Cmax in patients. All data were analyzed using 2way ANOVA of repeated measures (treatment and time). Upper panels: time course of glutamate changes (bins of 10 min). Lower panels: AUC. S-ketamine treatment led to significantly increased glutamate levels in rats at 1fold clinically efficacious Cmax (10 mg/kg), a dose which also shows efficacy in the forced-swim test (FST) and leads to increased locomotor activity (LA) in mice, mirroring psychotic effects in patients. Traxoprodil treatment significantly increased glutamate levels in rats at 3fold clinically efficacious Cmax (20 mg/kg), a dose showing efficacy in the FST but no psychotomimetic effects in LA in mice. Lanicemine, the clinically inactive compound, failed to increase glutamate levels in rats at up to 30fold clinically achieved exposures (90 mg/kg).

**Conclusions**

Here, we show for the first time, using glutamate voltammetry as a surrogate marker, that prefrontal disinhibition correlates with the clinical efficacy of NMDA-R antagonists. Circuit engagement in the PFC occurs only in the case of clinically efficacious NMDA-R antagonists such as S-ketamine and traxoprodil, and is absent in the case of the clinically ineffectivacious NMDA-R antagonist lanicemine. Furthermore, we show that the glutamate increase observed in the PFC of rats upon treatment with clinically efficacious NMDA-R antagonists is most likely not related to psychotomimetic side effects, but to antidepressant-like effects, as it is present in the case of traxoprodil and HNK, but absent in the case of lanicemine, none of which elicit psychotomimetic side effects. Taken together, the increase in glutamate levels in the PFC by clinically efficacious NMDA-R antagonists described here may be a valid predictor of antidepressant effects in patients.

**Conflict of interest:** All authors are employees of Boehringer Ingelheim.

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