Investigating Ketamine's Impact in Neuronal Excitability and Synaptic Activity for Translational Neuroscience Applications

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Abstract

Ketamine, a dissociative anesthetic with rapid-acting antidepressant effects, has demonstrated clinical efficacy in major depressive disorder (MDD) and other neuropsychiatric conditions. While its role as a noncompetitive NMDA receptor antagonist is well established, the systems-level mechanisms by which ketamine modulates neural circuits remain poorly defined.

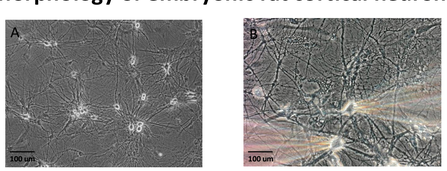
At therapeutic doses, ketamine selectively blocks NMDA receptors, reducing excitatory neurotransmission. Ketamine is thought to preferentially inhibit NMDA receptors on GABAergic interneurons, leading to disinhibition of excitatory neurons. Over time, this increased glutamate in the synaptic cleft and enhanced AMPA receptor activity are thought to drive synaptic plasticity—including elevated BDNF release, mTOR signaling, dendritic spine formation, and synaptogenesis—particularly in the prefrontal cortex. However, identifying a quantifiable and reproducible functional endpoint of ketamine's action remains essential for advancing both basic research and clinical translation.

In this study, we performed whole-cell patch-clamp recordings to assess spontaneous action potentials, miniature excitatory postsynaptic currents (mEPSCs) inhibitory postsynaptic currents (mIPSCs), and spontaneous excitatory postsynaptic currents (sEPSCs) in cortical neurons across a range of ketamine concentrations. These studies should provide insights into the mechanism of ketamine on network activity and excitatory-inhibitory balance. Such metrics are critical for evaluating therapeutic efficacy, optimizing dosing strategies, and understanding how drugs reshape cortical circuit dynamics in health and disease.

Methods

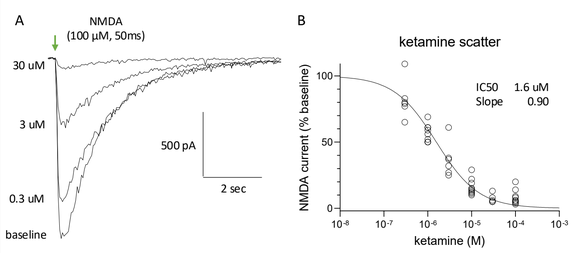
- Embryonic rat cortical neurons were plated at a low density (50-100 cells/mm2) on confluent monolayers of rat astrocytes.
- Whole cell patch clamp recordings were made using standard methods. The composition of external recording solution was: 140 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES pH 7.3. The composition of internal recording solution was: 120 mM K-gluconate, 20 mM KCl, 3 mM $MgCl_2$, 5 mM EGTA, 0.5 mM $CaCl_2$, 4 mM Na_2-ATP, 0.3 mM Li-GTP, 10 mM HEPES pH 7.3.
- Microelectrode array (MEA) recordings were made using the MaxWell MaxTwo high-density platform. Primary rat cortical neurons were co-cultured with astrocytes at a 2:1 ratio (10,000 neurons and 5,000 astrocytes per well).

Morphology of embryonic rat cortical neurons



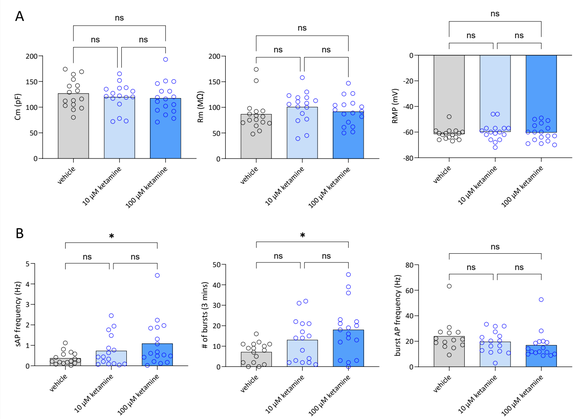
Photomicrographs of cultured embryonic rat cortical neurons at 25 DIV (A) and patched neurons at 26 DIV under a patch-clamp microscope (B).

Ketamine blocked NMDA receptor currents in primary rat cortical neurons



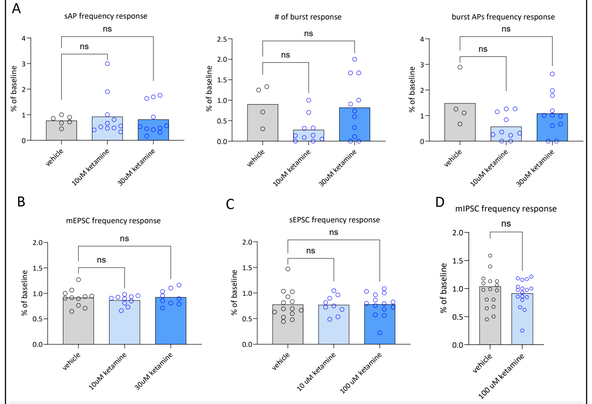
(A) Representative NMDA receptor currents from primary rat cortical neurons cultured for 2-4 weeks in vitro. NMDA was applied externally through a local puffer pipette to evoke inward currents. Ketamine was bath applied at the indicated concentrations. (B) Scatter plot showing the concentration–inhibition relationship for ketamine. The dose–response curve is averaged from n = 19 cells. IC_{50} and Hill slope values were 1.6 µM and 0.9, respectively.

Overnight ketamine treatment (24 hrs) increased spontaneous firing and bursting in primary rat cortical neuron cultures



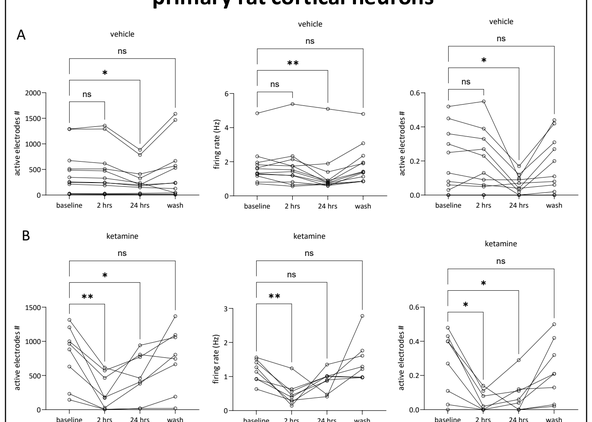
Primary rat cortical neuron were exposed to ketamine overnight, and sAPs were subsequently recorded using whole-cell patch-clamp electrophysiology. (A) Chronic ketamine treatment did not affect the passive properties of neurons (capacitance, input resistance, resting membrane potential). (B) Chronic ketamine treatment significantly increased spontaneous AP frequency relative to control. Data are presented as mean values; each group included recordings from 16-17 cells. Statistical analyses were performed using one-way ANOVA, with $p < 0.05$ considered significant. Ketamine (100 µM) vs. vehicle: p = 0.038 for # of APs and p = 0.012 for # of bursts.

Acutely applied ketamine did not alter functional endpoints in rat cortical neuron cultures



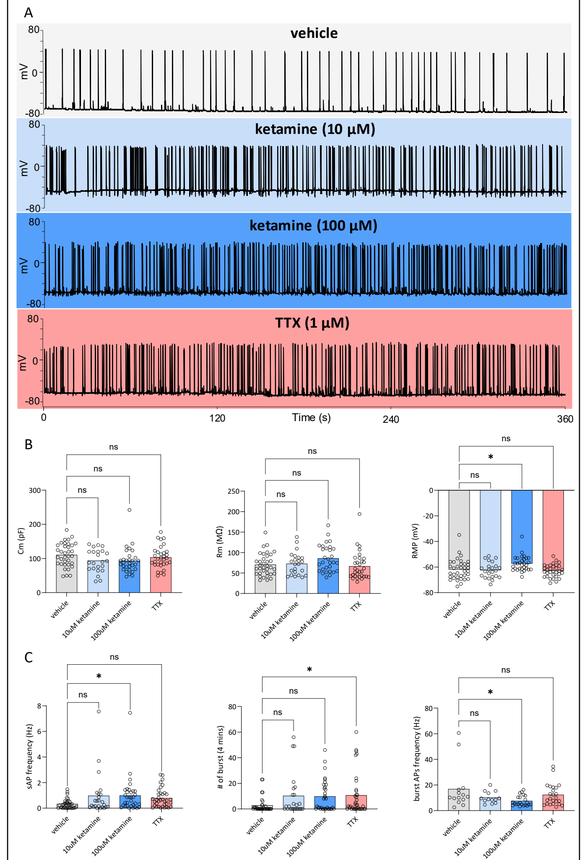
E18 cortical neurons were acutely exposed to ketamine. Endpoints measured were (A) spontaneous action potentials (sAPs), (B) miniature excitatory postsynaptic currents (mEPSCs), (C) spontaneous excitatory postsynaptic currents (sEPSCs), and (D) miniature inhibitory postsynaptic currents (mIPSCs) were examined with whole-cell patch-clamp recordings. Data are presented as mean values. Each condition included recordings from 6 - 18 cells from two to three independent culture cohorts, which produced qualitatively similar results.

Acutely applied ketamine decreased MEA activity in primary rat cortical neurons



Primary rat cortical neurons were exposed to ketamine for 2 or 24 hours. The number of active electrodes recorded using high-density MEA reflects neurons exhibiting spontaneous action potential firing. Each condition includes recordings from 6 - 10 MEA wells obtained from two independent culture cohorts, which yielded qualitatively similar results.

Overnight ketamine and TTX similarly enhance spontaneous neuronal activity in cortical neurons.



Overnight exposure to ketamine or tetrodotoxin increased spontaneous firing and bursting in rat cortical neurons (mean ± SEM, n = 24–36 cells; one-way ANOVA, $p < 0.05$). mEPSCs, mIPSCs, and sEPSCs were unchanged after ketamine treatment (data not shown).

Summary

1. Rat neuron cultures were used to interrogate the effects of ketamine on functional endpoints using electrophysiological recording methods (patch clamp and MEA).
2. Chronic ketamine treatment (24 h) increased the spontaneous AP firing rate without affecting mEPSCs, while acute application had no effect.
3. In contrast, MEA recordings revealed that acute ketamine reduced activity.
4. In vitro patch-clamp and MEA recordings could support psychiatric drug discovery, pending further validation.