Zanos *et al.* reply

REPLYING TO K. Suzuki *et al. Nature* **546,** http://dx.doi.org/10.1038/nature22084 (2017)

In the accompanying Comment[1], Suzuki *et al.* confirmed our previous findings[2] that the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine (HNK) does not functionally inhibit the NMDAR at concentrations relevant to its antidepressant actions recently reported in mice (that is, approximately 10 μM). We reported that 10 μM (2*R*,6*R*)-HNK, which equates to the maximum concentration of brain exposure (10.69 μM (ref. 2)) after intraperitoneal administration of a 10 mg kg^{-1} antidepressant dose of (2*R*,6*R*)-HNK in mice, robustly potentiated excitatory postsynaptic potentials in hippocampal slices without functionally inhibiting the NMDAR[2], as Suzuki *et al.* now confirm. Unless NMDAR inhibition is observed at 10 μM (2*R*,6*R*)-HNK, it is probably not of major relevance to the antidepressant actions of (2*R*,6*R*)-HNK. Notably, (2R,6R)-HNK administration also resulted in significant antidepressant actions at the dose of 5 mg kg^{-1} in mice, which would produce even lower concentrations of the metabolite in the brain than 10 μM (ref. 2). Furthermore, our field-potential electrophysiology experiments were conducted in the presence of the NMDAR antagonist AP5 (80 μM), demonstrating that the AMPAR-mediated synaptic potentiation we observed is independent of any capacity of (2*R*,6*R*)-HNK to inhibit the NMDAR.

We agree with Suzuki *et al.* that assessing higher concentrations of (2*R*,6*R*)-HNK on NMDAR-mediated miniature excitatory postsynaptic currents (NMDAR-mEPSCs) responses might be important in providing information for off-target effects of this metabolite, and their study indeed provides evidence for a modest inhibition (~37%) at the concentration of 50 μM. The absence of Mg^{2+} in the testing conditions may influence the apparent discrepancy between 37% inhibition of NMDAR-mEPSCs at the 50 μM concentration (for example, as is the case with memantine, which only inhibits NMDAR-mEPSCs in the absence of Mg^{2+}, and does not have antidepressant properties in humans[3]), and the previously reported lack of displacement of [^{3}H] MK-801 binding at less than 100 μM (ref. 4). It will be important to follow up on this finding with a full concentration response curve in order to determine the half maximal inhibitory concentration (IC_{50}) of (2*R*,6*R*)-HNK on NMDAR-mEPSC responses, and to compare to the IC_{50} of (*R*,*S*)-ketamine, under the same conditions using physiological levels of Mg^{2+}. Notably, we did not observe NMDAR-inhibition-associated psychostimulant, self-administration, drug discrimination and pre-pulse inhibition side effects of (2*R*,6*R*)-HNK at any dose, including up to 375 mg kg^{-1} (ref. 2).

The authors also observed decreased phosphorylation of the eukaryotic elongation factor 2 (eEF2) only at the concentration of 50 μM. Although this is the same high concentration at which (2*R*,6*R*)-HNK induced an inhibition of the NMDAR responses, these data do not provide direct evidence of a functional link between the moderate inhibition of NMDARs and the decrease in eEF2 phosphorylation. Indeed there are multiple mechanisms other than NMDAR inhibition that could be responsible for the observed eEF2 dephosphorylation[5–8]. It is also important to note that although direct application of ketamine[9] or the alternative NMDAR blocker MK-801 (ref. 10) to cultured neurons or hippocampal slices was previously reported to induce NMDAR-inhibition-dependent synaptic potentiation, MK-801 has repeatedly failed to induce ketamine-like sustained antidepressant effects in several animal tests[2,9,11,12]. It is therefore probable that synaptic strengthening as a consequence of NMDAR inhibition (proposed by Suzuki *et al.*) is not solely responsible for the antidepressant actions of ketamine. Indeed, the results of clinical trials indicate that several other NMDAR antagonists lack the full antidepressant actions of ketamine in humans[13].

Suzuki *et al.* also propose that NMDAR inhibition by (*R*,*S*)-ketamine is responsible for the initiation of antidepressant responses (that is, the acute actions), and HNK metabolites mediate the long-term antidepressant effects of ketamine through continued NMDAR inhibition. This hypothesis is not supported by our pharmacokinetic measurements of ketamine and its metabolites in the brain of mice following intraperitoneal administration of (*R*,*S*)-ketamine, which show that both ketamine and its metabolites peak at similar early time points, have a similar short half-life, and are below detectable levels by four hours after treatment[2].

In conclusion, the data presented by Suzuki *et al.* further support an NMDAR-inhibition-independent mechanism of antidepressant action of the ketamine metabolite (2*R*,6*R*)-HNK at physiologically relevant concentrations. Although development of (2*R*,6*R*)-HNK as an antidepressant drug, which does not induce NMDAR-mediated side effects, can proceed independently of target identification, it remains important to identify its primary pharmacological target at antidepressant-relevant concentrations (~10 μM).

Author Irving W. Wainer has chosen not to be listed on this Reply.

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