Interactions between ketamine and opioid receptors in nonhuman primates

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Background

Ketamine is an NMDAR antagonist that has rapid-acting antidepressant effects[1,2]. Despite ketamine's known action at the NMDAR, it also acts as an agonist at μ and κ opioid receptors with slightly lower binding affinity[3]. Previous studies in humans have demonstrated that activation of opioid receptors is necessary for ketamine's antidepressant effects[4], and work in rodents supports these findings[5–8]. No studies have been performed in nonhuman primates (NHPs) investigating ketamine's interactions with the opioid receptor system. NHPs are particularly well-suited for preclinical studies to investigate and develop treatments for psychiatric illnesses because NHPs have similar stress-related behaviors, social structures, and prefrontal cortical brain development to that of humans.

Methods

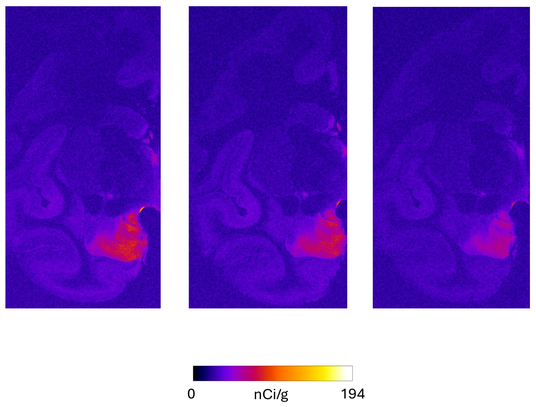
Autoradiography: Using flash-frozen brain tissue slices from the amygdala, nucleus accumbens, and thalamus from three rhesus macaques, we conducted receptor autoradiography to investigate ketamine's ability to bind to μ and κ opioid receptors. Regions of interest were determined by inspection based on the Paxinos atlas[9]. To assess ketamine binding to mu opioid receptors, tissue sections were incubated with 5 nM [^{3}H]DAMGO, a μ opioid receptor-selective ligand, in the presence of absence of 1 or 10 μM ketamine. To assess ketamine binding to κ opioid receptors, tissue sections were incubated with 15 nM [^{3}H]U-69,593, in the presence or absence of 1 or 10 μM ketamine. Slides were placed into a Hypercassette™ covered by a BAS-SR2040 phosphor screen (FujiFilm; GE Healthcare). The slides were exposed to the phosphor screen for 3–5 days and imaged using a phosphor imager. ROIs were manually identified based on a digital atlas[10,11], and signal intensity was quantified using ImageJ. Data were analyzed using paired, one-tailed t-tests, and analyses were performed with GraphPad Prism 9.

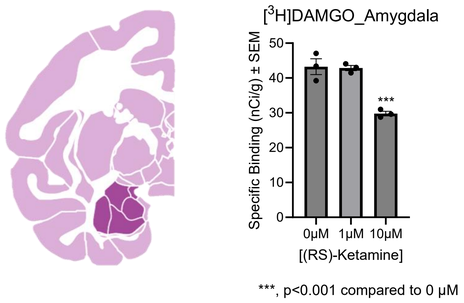
Positron Emission Tomography (PET): Three animals received 90-minute baseline scans of [^{18}F]FE-DPN, which is an opioid receptor radioligand. Two animals received multiple ketamine challenge scans (2.25 mg/kg CRI, 2 boluses of 5 mg/kg IM ketamine 5 minutes before and 60 minutes into the scan, and 5 mg/kg IV bolus plus 15 mg/kg CRI). One animal also received a naltrexone pretreatment scan. A population template was created using baseline scans. Average time activity curves for the amygdala and cerebellum were extracted from each animal that received ketamine challenge scans to generate the ratio graphs. Area under the curve (AUC) of these graphs was used to estimate radioligand displacement.

Results

10 μM of racemic ketamine significantly reduced binding of the μ opioid receptor ligand, [^{3}H]DAMGO, in the amygdala and the κ opioid receptor, [^{3}H]U69,593 in the medial claustrum (both p's < 0.001). 10 μM of racemic ketamine tended to reduce [^{3}H]U69,593 binding in the amygdala (p = 0.061). 1 μM ketamine did not significantly reduce [^{3}H]DAMGO or [^{3}H]U69,593 binding in the amygdala (both p's ≥ 0.10), but it did reduce [^{3}H]U69,593 binding in the medial claustrum (p = 0.0073). [^{18}F]FE-DPN image analysis demonstrated specific binding that was markedly decreased by naltrexone pretreatment. High, anesthetic doses of ketamine resulted in a 5-10% decrease in [^{18}F]FE-DPN binding.

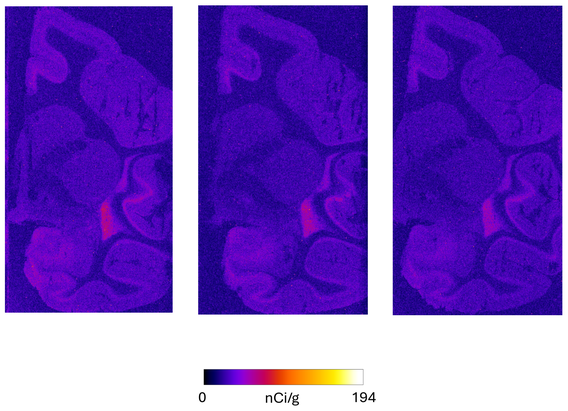
μ Opioid Receptor Binding

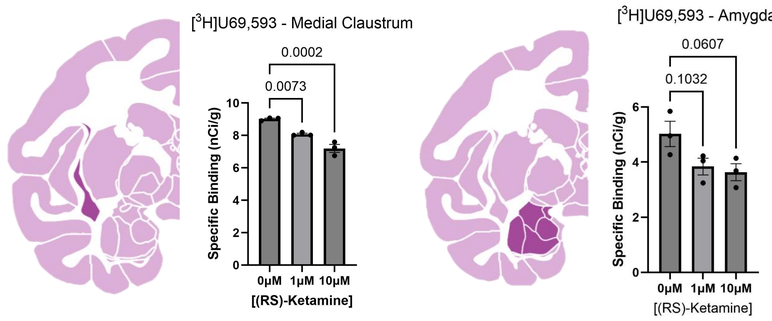




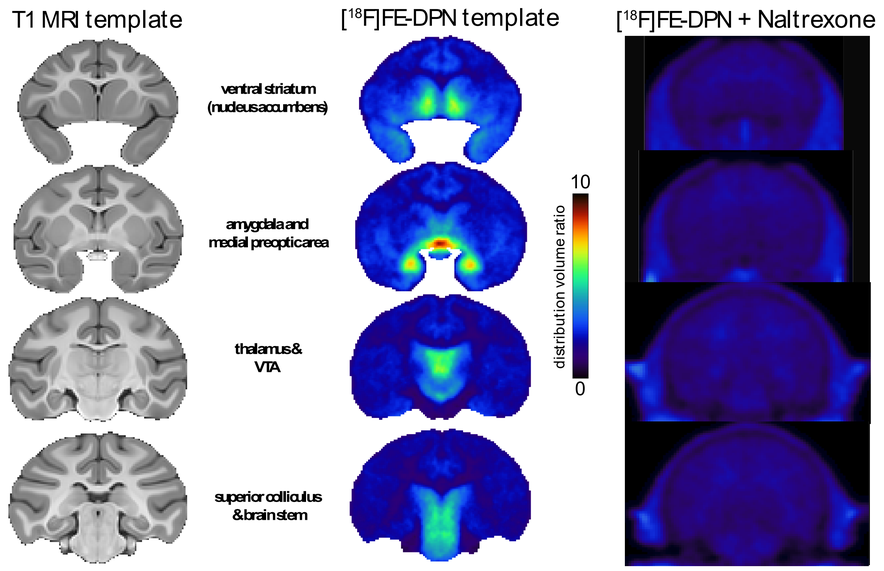
***, p<0.001 compared to 0 μM

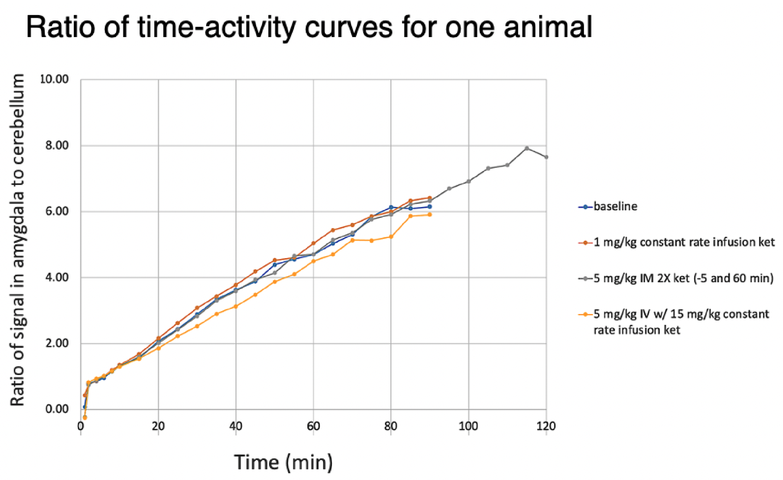
κ Opioid Receptor Binding





[^{18}F]FE-DPN Results





Conclusions

Autoradiography data demonstrated that ketamine generally displaces OR-selective radioligands at high concentrations but not at lower concentrations thought to be relevant to doses used to treat depression. More specifically, pharmacokinetic data from our lab estimates CSF ketamine concentrations of 0.5 μM 15 minutes after a dose of 1 mg/kg IM ketamine, which is similar to the lower concentration of ketamine (1 μM) used in the autoradiography studies. Additionally, [^{18}F]FE-DPN is a promising PET ligand for imaging the primate opioid receptor system. The naltrexone blocking scan confirmed that the [^{18}F]FE-DPN binding observed in the primate brain is specific to opioid receptors. However, the effects of ketamine in displacing [^{18}F]FE-DPN binding were less robust when compared to the autoradiography findings as displacement was observed for only a high, anesthetic dose. These data together suggest that ketamine interacts with the rhesus macaque brain in a dose-dependent manner.

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