Establishing the Parameters of Ketamine Administration to Assess Effects on Pathological Anxiety in Non-human Primates

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### Background
Ketamine is an anesthetic that has rapid-acting antidepressant effects when administered in subanesthetic doses. Although ketamine has been approved by the FDA to treat depression, the mechanism of its antidepressant action remains unknown and warrants further investigation. Most of the preclinical studies on ketamine have been done in rodent models. However, non-human primate (NHP) models are critical to bridge the preclinical and translational research gap between rodents and humans. Anxiety disorders are often comorbid with depression and the Kalin Lab has a well-established NHP model of pathological anxiety, which is ideally suited to further investigate mechanisms underlying ketamine's effects and to translate these findings to humans. The aim of the studies presented here is to identify a suitable subanesthetic dose of ketamine for use in our NHP model.

### Dose Response Study Design
The goal of this study was to identify a subanesthetic dose of ketamine that is analogous to that used to treat depression in humans. Six rhesus macaques were used (3 male, 3 female; about 1.5 years of age). Each animal received vehicle, 0.5 mg/kg ketamine, and 1.0 mg/kg ketamine IM with a two-week interval between treatments. Treatments were counterbalanced for order. Animals were removed from their home cage, injected, and placed alone in the test cage. Behavioral data was collected for 90 min. A human intruder entered the room at 15, 30, 60, and 90 minutes after injection and stared at the animal for 3 minutes. Blood and CSF were collected at the end of the paradigm. Behavior was scored in five minutes bins by two independent raters.

### Pharmacokinetic Study Design
Four rhesus macaques were used in the pharmacokinetic (PK) study (2 male, 2 female; about 1.5 years of age). Each animal received an IM injection of 1.0 mg/kg ketamine. Blood was sampled at 5, 15, 30, 45, 60, 90, 120, 240 minutes, and 24 hours post-administration. A single CSF sample was collected 15 minutes after injection. Samples were analyzed for the concentration of R and S-ketamine, norketamine, hydroxynorketamine, and dehydroxynorketamine. At the time of the assay, standards were unavailable for the metabolites, so only relative area under the curve values were generated.

### Dose Response Study Data

#### Results:
Ketamine has dose-dependent effects on a variety of behaviors including motion related behaviors and vocalizations. These effects resolve within approximately 45 minutes after IM administration.

### Pharmacokinetic Study Data

#### Results:
Ketamine has a half-life of approximately 30 minutes and is fully cleared from the body 4 hours post IM administration. Metabolites of ketamine are cleared from the body by 24 hours.

### Conclusions
The subanesthetic dose of ketamine that was chosen for further testing of effects on anxiety was 1.0 mg/kg. This dose had short term effects on behavior that did not extend beyond 45 minutes, and there were no signs of significant sedation. The PK data are consistent with the time course of the behavioral data, as they show that ketamine has a plasma half-life of approximately 30 minutes in NHPs.

### Ongoing Study—Assessing Ketamine’s Effects on Anxiety
We are currently analyzing data from a study assessing the effects of ketamine on anxiety-like responding in NHPs. The study uses a within-subjects crossover design to test the effects of 1.0 mg/kg ketamine vs vehicle on anxious behaviors in 11 animals (6 males, 5 females). Twenty-four hours after ketamine or vehicle treatment, each animal is placed in a human intruder paradigm (HIP) to assess different facets of anxiety. The 24-hour time point corresponds to when the antidepressant effects of ketamine are observed clinically. We hypothesize that animals that received ketamine treatment will display a reduction in anxious-like behaviors in the HIP.