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Gender Differences In The Metabolism And Antidepressant Effects Of Ketamine

Emily Hugo
University of Alabama at Birmingham

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by

EMILY HUGO

ELIZABETH GARDNER, COMMITTEE CHAIR
JASON LINVILLE
LORI MCMAHON

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MASTER OF SCIENCE IN FORENSIC SCIENCE

ABSTRACT

Ketamine is a dissociative anesthetic that interacts with the glutamatergic system, acting as an *N*-methyl-*D*-aspartate (NMDA) receptor antagonist. At higher doses, ketamine produces negative psychotomimetic side effects, including hallucinations and delirium, leading to its abuse in the 1980s and 90s. Recently, it has been determined that at sub-anesthetic doses, it is a fast-acting antidepressant. Because ketamine elicits fast and long-lasting antidepressant responses in patients, it is being studied as a possible treatment option for those with major depressive disorder and treatment-resistant depression.

Further, studies have shown that men and women respond to ketamine treatments differently, especially in long-term treatment, suggesting that gonadal hormones may play a role in how ketamine interacts with the brain. Dr. Lori McMahon, Allie Widman, and Nateka Jackson in the Cell, Developmental, and Integrative Biology Department at the University of Alabama at Birmingham are studying the differences in male and female responses to ketamine. The toxicological results from this graduate research project will be combined with the behavioral observations of the rats by the McMahon group to better understand the differences in how the genders metabolize ketamine.

For this research, female rats were ovariectomized (OVX) and half received estrogen (OVX/E2). All of the rats received injections of ketamine and were sacrificed at

two timepoints, either 0 or 30 minutes. Ketamine and norketamine (NK) were quantified in biological tissue collected from the cerebellum, prefrontal cortex (PFC), hippocampus, liver, and plasma of the rats.

The results of this study indicate that ketamine is quickly distributed to the brain, specifically in the PFC, and is cleared from the tissues by 30 minutes. The NK concentration was greatest in the tissues at time 30 minutes, particularly in the PFC and liver. The main difference between the OVX and OVX/E2 rats was the amount of NK at time 30 minutes. The OVX/E2 rats had more NK in the tissue samples, however, the difference is not statistically significant.

Future studies can include quantitating other metabolites, such as hydroxynorketamine (HNK), to better understand how genders metabolize ketamine differently. Further, rats could be given multiple doses to evaluate the effects of ketamine over long-term treatment.

Keywords: Ketamine, Norketamine, Antidepressant, Metabolism, Gender Differences, NMDA antagonist

ACKNOWLEDGMENTS

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TABLE OF CONTENTS

Page

LIST OF FIGURES

INTRODUCTION

Ketamine (2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone) (Figure 1) is a non-barbiturate dissociative anesthetic and glutamatergic psychedelic and is used primarily as a veterinary and pediatric anesthetic.[1] More recently, it has been studied as a potential fast-acting antidepressant.[2,3] American chemist Calvin Stevens first synthesized ketamine in 1962 as a replacement for the anesthetic phencyclidine (PCP). While it is structurally similar to PCP, ketamine is less potent, has a faster onset and shorter duration of action, is safer to use, and has fewer negative side effects.[1,4] The Food and Drug Administration (FDA) approved ketamine for human and animal use in 1970, and it was used as a surgical anesthetic on soldiers during the Vietnam War.[1] Ketamine is mainly imported into the United States from Mexico and India and is distributed under the brand names Ketalar, Ketaset, Ketajet, Ketavet, Vetamine, Vetaket, and Ketamine Hydrochloride injection.[5]

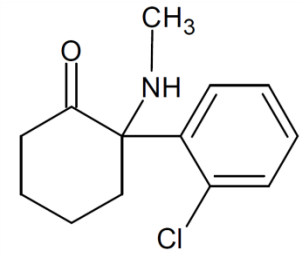

Figure 1. The chemical structure of ketamine

At high doses, ketamine produces psychotomimetic effects, including hallucinations and delirium.[6] Users can go through the "K-hole", where they feel disconnected and can have an out-of-body, near death experience.[5] Ketamine abuse rose

during the 1980s and 1990s, appearing in the dance club scene under the street names Special K, K, Kit Kat, and Cat Valium.[5] In August 1999, the Drug Enforcement Administration (DEA) placed ketamine on the United States Controlled Substances list as a Schedule III drug.[7] Although ketamine is usually taken alone, it has been mixed with MDMA, amphetamine, methamphetamine, cocaine, and carisoprodol.[5]

Use as Antidepressant in Humans

Due to its rapid onset (2-24 hours post-infusion) of antidepressant effects[8], ketamine is an attractive medication for treatment-resistant depression (TRD), diagnosed in patients that fail to respond to at least two other antidepressant treatments.[9] Traditional antidepressant medications act on the serotonergic and noradrenergic systems. They can take weeks to months to produce a response, leaving the patient susceptible to severe depressive symptoms and at risk for suicidal behavior.[10] On the other hand, drugs that interact with the glutamatergic system elicit fast antidepressant responses in patients. Ketamine is an antagonist of the *N*-methyl-*D*-aspartate (NMDA) receptor in the glutamatergic system.[11]

In the 1990s, scientists began studying ketamine as a potential treatment for depression[3], since preclinical research had shown that NMDA antagonists were effective in animal models of depression.[12] In 2000, Berman et al.[3] first demonstrated that ketamine at low doses has a potential to be used as an antidepressant medication in individuals who exhibited major depressive episodes. Patients (n=7) were administered either a 0.5 mg/kg dose of ketamine hydrochloride or a saline solution on two days at least one week apart. Overall, the ketamine treatment significantly decreased depressive

symptoms in the individuals and half of the patients showed 50% or greater improvement in their symptoms over the three-day period following the infusion.

Zarate et al.[13] conducted a similar experiment but evaluated the effects of ketamine on patients with TRD up to 7 days post-infusion. Individuals (n=18) received an intravenous infusion of 0.5 mg/kg ketamine hydrochloride or a saline solution on two days separated by one week. Seventy-one percent of the subjects receiving the ketamine treatment experienced a significant response within 110 minutes post-infusion as compared to those who received the placebo. The antidepressant effects lasted throughout the 7 days for 35% of the initial 71% experiencing a response. Zarate concluded that ketamine had a high efficacy and quickly produced antidepressant effects. Further, the effects from one injection may be maintained for up to one week or longer.

Long-term Effects

To better understand the long-term effects of ketamine, Murrough et al.[14] designed a study to determine if the antidepressant effects of ketamine could be prolonged and maintained in patients with TRD. During the study, 24 patients received up to six intravenous infusions of ketamine (0.5 mg/kg), given three times a week over a 12-day period. The 70% of the patients who responded positively to the ketamine treatments were monitored for up to 83 days to determine the length of time it took for the symptoms of depression to reappear. Four participants did not relapse over the course of the study. For the remainder, the median time to relapse was 18 days. The results demonstrated that ketamine has rapid antidepressant effects on individuals, and the effects are sustained when the patient receives multiple infusions. The study linked a

rapid response to the first ketamine infusion to a sustained response resulting from the subsequent infusions.

Singh et al.[8] conducted a study that compared the responses from two versus three weekly doses of ketamine (0.5 mg/kg) over four weeks. Both the two-dose and three-dose regimens produced similar antidepressant results.

Ketamine Enantiomers

Ketamine has a chiral center and has two enantiomers: *R*-ketamine and *S*-ketamine. It is typically administered as a racemic mixture.[15] However, it is not definitively known which enantiomer or its metabolite contributes more to the antidepressant effects, especially for a sustained response. Oye et al.[16] found that *S*-ketamine is more potent and has a five times greater affinity for the NMDA receptor than *R*-ketamine in the human brain, but it also produces more negative psychotomimetic side effects. Other studies, including Zhang et al.[17] and Yang et al.,[15] have concluded that the *R*-ketamine enantiomer elicits a faster, more potent, and longer-lasting antidepressant response than *S*-ketamine.

Ketamine is metabolized in the liver in both animals and humans.[4] The primary metabolic route is *N*-demethylation by cytochrome P450 enzymes[18] to form the major active metabolite norketamine (NK).[4] The NK can be hydroxylated to form six diastereomeric hydroxynorketamine (HNK) compounds or transformed into dehydronorketamine (DHNK).[19] Ketamine can also be hydroxylated to form two diasteromeric hydroxyketamine (HK) compounds.[19] The metabolic pathway for these compounds is shown in Figure 2.

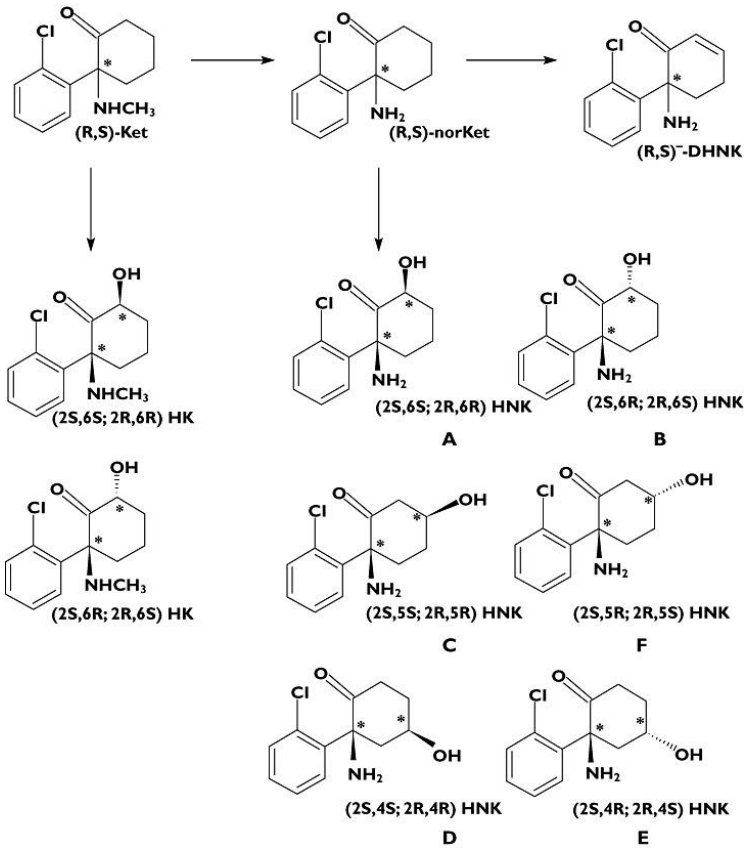

Figure 2. Metabolic pathway of ketamine and NK.[19] From "Simultaneous population pharmacokinetic modelling of ketamine and three major metabolites in patients with treatment-resistant bipolar depression" by Zhao, X., et al., 2012, *British Journal of Clinical Pharmacology*, 74(2), p. 305. Reprinted with permission.

Identification of the Active Antidepressant Metabolite

Several studies have been conducted to determine how the metabolites of ketamine contribute to its antidepressant effects. Zhao et al.[19] quantified ketamine and some of the metabolites in human plasma following *R,S*-ketamine (0.5 mg/kg) infusions. Ketamine was quickly metabolized within 4 hours, with a peak concentration of NK at 80 minutes, and then steadily decreased through the 230-minute point. Even though NK was the primary metabolite, other metabolites may be responsible for the antidepressant effects of ketamine. Both *R,S*-DHNK and (2*S*,6*S*; 2*R*,6*R*)-HNK were present in the

plasma samples for 24-48 hours after administration, and HK was below the limit of quantitation (LOQ).

Salat et al.[20] eliminated DHNK as the active antidepressant. They observed the effects of ketamine, NK, and DHNK on several receptors, ion channels, and transporters in mouse brains. The effects of dosing mice with racemic ketamine, racemic NK, or DHNK were evaluated by conducting various behavioral and receptor binding tests. They concluded that ketamine and NK most strongly interacted with the NMDA receptors and reduced immobility in the behavioral tests with the minimum effective dose of 10 mg/kg and 50 mg/kg for ketamine and NK, respectively. However, DHNK did not affect immobility even at the highest dose of 50 mg/kg.

Zanos et al.[21] have suggested that (2*S*,6*S*;2*R*,6*R*)-HNK may be an active antidepressant metabolite. They dosed mice with deuterated ketamine, which hindered ketamine from metabolizing to (2*S*,6*S*;2*R*,6*R*)-HNK, and they did not observe any antidepressant effects. They concluded that the metabolism of ketamine, specifically the metabolism of ketamine to (2*S*,6*S*;2*R*,6*R*)-HNK, may be required for antidepressant effects. Further, they dosed mice with either (*2S*,6*S*)-HNK or (2*R*,6*R*)-HNK and determined that the (2*R*,6*R*)-HNK enantiomer elicited greater antidepressant responses in the mice. This further supports the claim that *R*-ketamine may be responsible for the antidepressant effects of ketamine. However, Shirayama et al.[22] argues that HNK is not responsible for contributing to the antidepressant effects of ketamine. They directly compared the antidepressant effects of (*R*)-ketamine, (*R*)-NK, and (2*R*,6*R*)-HNK by injecting rats with one of the compounds. They observed that only the (*R*)-ketamine

elicited antidepressant responses after a single injection, suggesting that the parent drug itself may be required to improve depression.

An alternative explanation for the conflicting results relative to the active and inactive antidepressant metabolites is the lack of standardization of doses, length of treatment, and type of behavioral test conducted to evaluate the antidepressant effects of the various compounds on the animals or humans.

Gender Differences in Ketamine Response

Some research indicates that male and female rats metabolize and respond to ketamine differently. In the study done by Zanos et al.[21], ketamine had more potent antidepressant effects in the female mice. Further, the results showed that the genders metabolized ketamine differently, as the concentration of (2*S*,6*S*;2*R*,6*R*)-HNK was three-times higher in the female mice than the male mice. Since women are twice as likely to experience depression as men, especially during the reproductive years,[23] gender differences may be significant when evaluating the efficacy of ketamine as an antidepressant medication.

In female rats, the estrous cycle lasts four days and consists of four periods: proestrus, estrus, metestrus, and diestrus. Ovulation occurs during the proestrus and estrus periods.[24] The gonadal hormones peak during the proestrus period and are lowest during the diestrus period.[25] The stage can be determined by examining the cells present in vaginal smears.[26] Wright et al.[25] conducted a study to better understand the effects and abuse potential of low-dose intermittent ketamine treatment in male and female rats. The rats received self-administered ketamine infusions (0.1 mg/kg) when they selected the

active nose poke in the operant chamber, and the ketamine would be delivered to the rat through a catheter. Males were able to self-administer ketamine every fourth day while intact females received ketamine on certain days in the estrus cycle, depending on their levels of gonadal hormones. The rats' addiction behaviors were studied by reducing the amount of time they were able to receive ketamine infusions. The results showed that the female rats in the diestrus stage did not become as addicted to ketamine as the male and proestrus-trained female rats, demonstrating that the female's response to ketamine depended on the stage of the estrus cycle and the hormones at the time of administration.

Carrier et al.[27] injected male and female rats with ketamine (0, 2.5, 5, or 10 mg/kg) and found that the female rats had a greater sensitivity to the lower dose of ketamine (2.5 mg/kg) than the male rats. To study the effects of ketamine in conjunction with estrogen, ovariectomized (OVX) female rats were injected with estrogen benzoate (0, 2, or 10 µg). All the rats received injections of either 0.0 or 2.5 mg/kg of ketamine. Only the females dosed with estrogen benzoate were sensitive to the lower dose of ketamine (2.5 mg/kg).

Further, Thelen et al.[28] injected mice with ketamine (3, 5, or 10 mg/kg) every day for 21 days. Following the 21-day treatment, opposite effects were observed in each gender. The male mice displayed antidepressant effects while the female mice showed anxiety-like and depressive-like effects. These studies demonstrate that gonadal hormones in females may affect how rats respond to ketamine, especially over longer periods of time.

Previous Collaboration Research

This project builds on previous work by Elise Erb[29] who studied the gender differences in ketamine metabolism in collaboration with Dr. Lori McMahon's group in the Cell, Developmental, and Integrative Biology Department at the University of Alabama at Birmingham. Erb quantitated ketamine and NK in male and OVX female rats sacrificed at time 0, 30 minutes, and 3 hours following a ketamine injection (10 mg/kg). Ketamine was most abundant at time 0 and was essentially cleared from the tissues by 3 hours. The brain samples had higher concentrations than the plasma and liver at time 0, which indicates that ketamine reaches the brain quickly. The NK was only detected in the male liver samples at time 0. At time 30 minutes, the NK was still not detected in the plasma, and in the remaining samples, the concentration of NK was less than 2 ng/g of sample. The male hippocampus and liver samples had the highest amount of NK. By 3 hours, the NK was below the LOQ in all samples. Erb concluded that there was no significant difference between the male and OVX female rats. In this thesis research, ketamine and NK were quantitated in OVX and OVX/E2 female rats following a ketamine injection to better understand the differences in how males and females respond to this drug.

MATERIALS AND METHODS

Sample Collection

Adult female rats were ovariectomized (OVX). Two weeks following the ovariectomy, half of the rats received two subcutaneous injections of 10 μg/250 g estrogen (OVX/E2), separated by 24 hours. Fourteen OVX rats and fourteen OVX/E2 rats received intravenous injections of 10 mg/kg of ketamine. The OVX/E2 rats received the ketamine injection 24 hours following the second estrogen injection. Seven from each set were sacrificed at 0 and 30 minutes. Biological samples including plasma, liver, cerebellum, prefrontal cortex (PFC), and hippocampus were collected from each rat. Two control OVX/E2 rats were injected with a saline solution instead of ketamine and were sacrificed at 0 minutes. The biological samples were placed in polypropylene tubes and stored at -80°C until analysis.

Sample Preparation

This ketamine and NK extraction was adapted from the method developed by Erb.[29]

After the samples thawed, they were transferred into 2.0 mL polypropylene tubes. The liver and brain samples were weighed before 10-15 metal beads (2.4 mm) were added to each tube. To all biological samples, 500 μL of deionized water and 50 μL of the internal standard, ketamine-d_4 (100 mg/mL), were added. Each tube was vortexed until the sample was completely homogenized, and then the sample was centrifuged in an

Eppendorf Minispin centrifuge for 15 minutes at 12.5 x 1000 rpm. After centrifugation, the aqueous layer was transferred to a new 2.0 mL polypropylene tube containing 1 mL of acetonitrile to precipitate the proteins. The samples were vortexed for one minute and centrifuged again for 15 minutes at 12.5 x 1000 rpm. The aqueous layer was transferred to a test tube containing 2 mL of 0.1 M sodium acetate buffer at pH 5. The sodium acetate buffer was prepared by mixing a 0.1 M solution of acetic acid and a 0.1 M solution of sodium acetate at a ratio of 1:2.39.

Analyte Extraction

The sample preparation and analyte extraction technique was adapted from a ketamine extraction method from DPX Technologies. Solid-phase extraction dispersive pipette tips (5 ml, 5S-5TF25-02-030-050-5B DPX Technologies) were used to extract ketamine and NK from the biological samples. The pipette tip first was conditioned by aspirating and dispensing 3 mL of methanol and then 3 mL of deionized water. To begin the extraction process, the sample in the sodium acetate buffer was aspirated into the pipette tip. After approximately 15 seconds, the solution was dispensed back into the test tube. This step was repeated four times. Next, 2 mL of the sodium acetate buffer was aspirated and dispensed once, and then 2 mL of methanol was aspirated and dispensed one time. Finally, 3 mL of 3-5% ammonium hydroxide buffer solution in acetonitrile at pH 10 was aspirated and dispensed once. This final solution contained the analytes of interest.

The sample tubes were dried down under a nitrogen gas stream in an Organomation Associate's N-EVAP™112. Once dry, the sample was dissolved in 100

μL hexane and transferred to 200 μL microvials for GC/MS analysis using the Agilent 6890N Network GC system/5975 inert Mass Selective Detector with selected ion monitoring. The target ions used for quantitation for NK, ketamine, and ketamine-d_4 were 166, 180, and 184, respectively. The qualifier ions for ketamine were 138 and 152 and for NK were 131 and 195.

Quantitation and Analysis

A calibration curve to quantitate ketamine and NK in the unknown biological samples was created at least once a week. The calibration samples were prepared at 0.5, 1, 3, 5, 10, and 15 ng/μL using ketamine and NK standards (1.0 mg/mL, Cerilliant). Fifty μL of the internal standard, ketamine-d_4 (100 μg/mL, Cerilliant), was added to each sample. The target analytes were extracted using the previously described extraction procedure used for the experimental samples. A line of best fit for the data was created using Microsoft Excel. Positive control samples (1 ng/μL) were run with the animal biological samples as a check on the quality of the calibration curves.

RESULTS

The average retention times for NK, ketamine, and ketamine-d_4 were 8.8, 9.9, and 9.8 minutes, respectively. The ions 131, 166, and 195 were monitored for NK, and 138, 152, 180, and 184 were monitored for ketamine and ketamine-d_4. A representative chromatogram and mass spectra are shown in Figure 3.

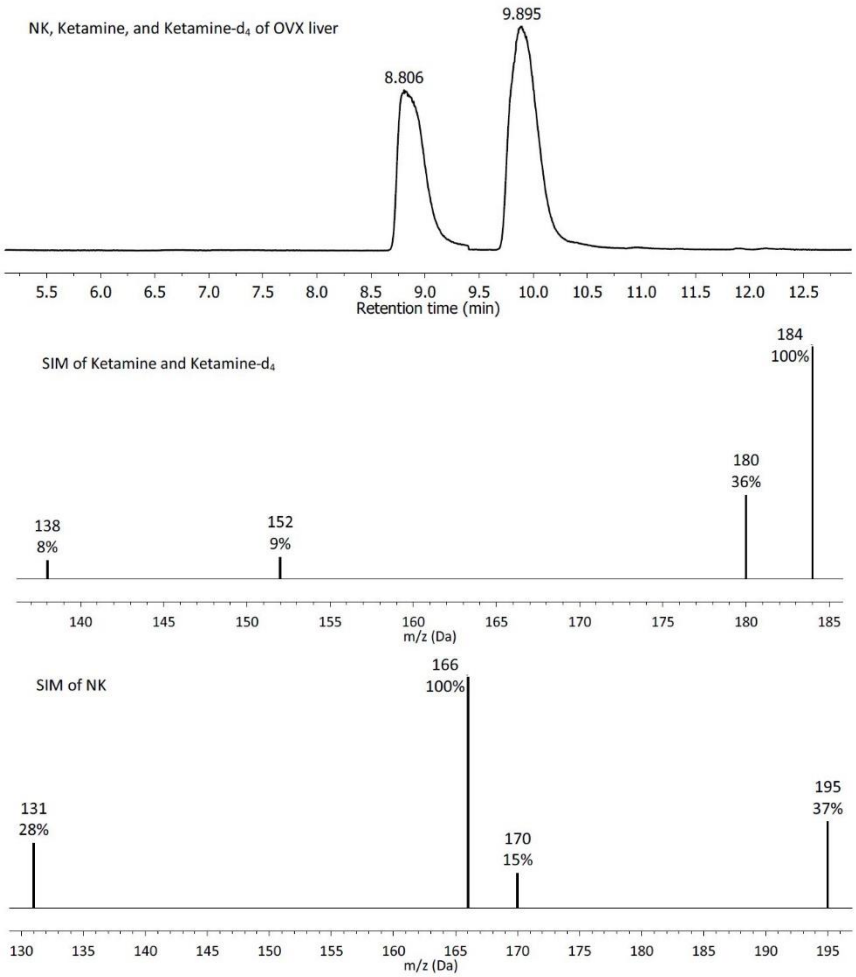

Figure 3. Representative chromatogram and mass spectra for each analyte. The chromatogram is from the liver of an OVX female sacrificed at 30 minutes. The retention time of NK was 8.806 minutes, and ketamine and ketamine-d_4 co-eluted at 9.895 minutes. The top mass spectrum is from the OVX liver at 30 minutes, and the bottom spectrum was obtained from the PFC of an OVX female sacrificed at 30 minutes.

Ketamine and NK in the samples were quantified (ng/µL) using 6-point calibration curves (0.5, 1, 3, 5, 10, and 15 ng/µL). All calibration curves were linear, and the R^2 values ranged from 0.99 to 0.9998 for ketamine and from 0.92 to 0.999 for NK. These calibration curves were also used to determine the limits of quantitation for ketamine and NK, which was 0.5 ng/µL for both. The calibration curves were created at least once a week. A representative curve is shown in Figure 4.

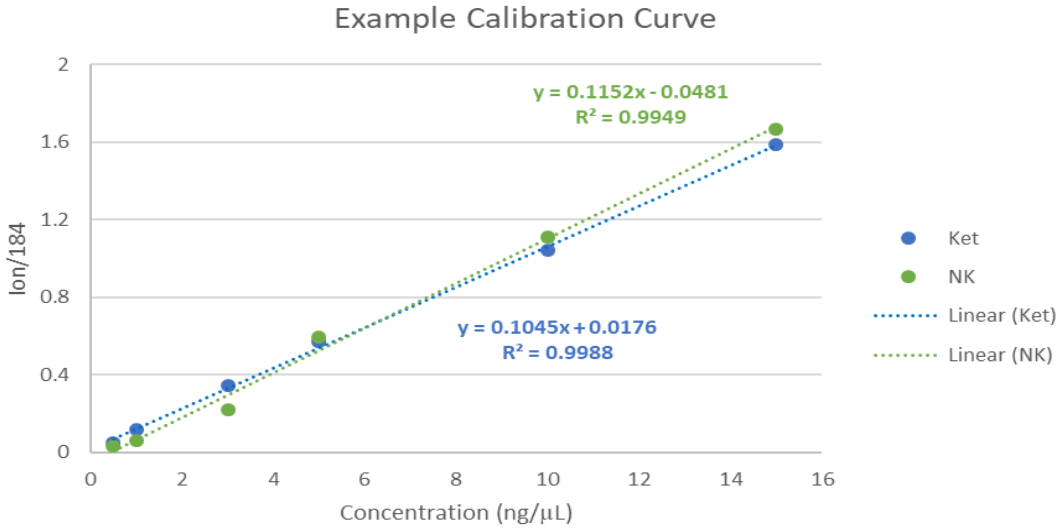

Figure 4. Example Calibration Curve. These calibration curves were used to calculate the concentrations (ng/µL) of ketamine (blue) and NK (green) in the biological samples.

The ketamine and NK concentrations calculated from the calibration curve were converted to µg/g tissue to account for sample mass, using the mass correction formula (Equation 1). Tables showing the masses of the samples are included in Appendix B.

$$\text{corrected mass} = \frac{100\ \mu\text{L} * \text{concentration}\ \left(\frac{\text{ng}}{\mu\text{L}}\right) * \left(\frac{\mu\text{g}}{1000\ \text{ng}}\right)}{\text{g tissue}} \qquad \text{(Eq. 1)}$$

The amount of ketamine and NK (µg/g tissue) in the biological samples for the OVX and OVX/E2 rats sacrificed at times 0 minutes and 30 minutes are shown in

Figures 6 and 7, respectively. A reference code that correlates the rat number from the behavioral study to the sample number in this research is shown in Appendix C. If the ketamine or NK was not detected in the sample, the sample is marked with an "**N**". If the analyte was present but was below the LOQ, the sample is marked with a "**P**". An example of a peak that was present but below the LOQ is shown in Figure 5. If the analyte was above the LOQ, the sample is marked with an "**A**". Finally, if the data could not be collected for the sample, that sample was not included in these figures. The data could not be collected for one of two reasons: (1) the frit in the extraction pipette tip broke, or (2) the internal standard indicated poor data.

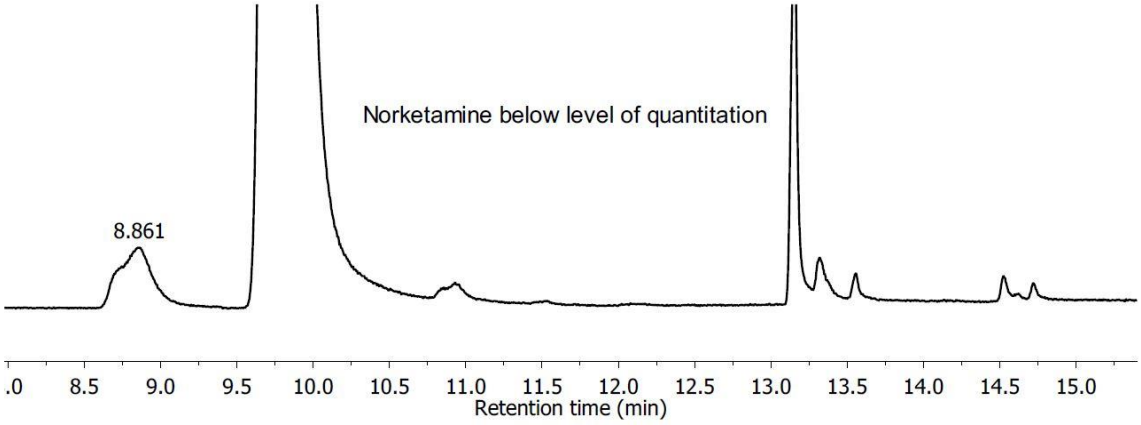

Figure 5. Example chromatogram that shows a NK peak (8.861 min.) that is present but below the LOQ (0.5 ng/µL).

The ketamine injection in animals 3.1 (OVX/E2, 0 min.) and 6.1 (OVX, 30 min.) were reported as likely missing the vein. This is supported by the low values of ketamine and NK detected in these tissues. Similarly, the values in the samples from animal 2.1 (OVX, 0 min.) were similar to animals 3.1 and 6.1 and were unlike the other OVX, 0 min. sample set. Therefore, these three animals were not included in Figures 6 or 7.

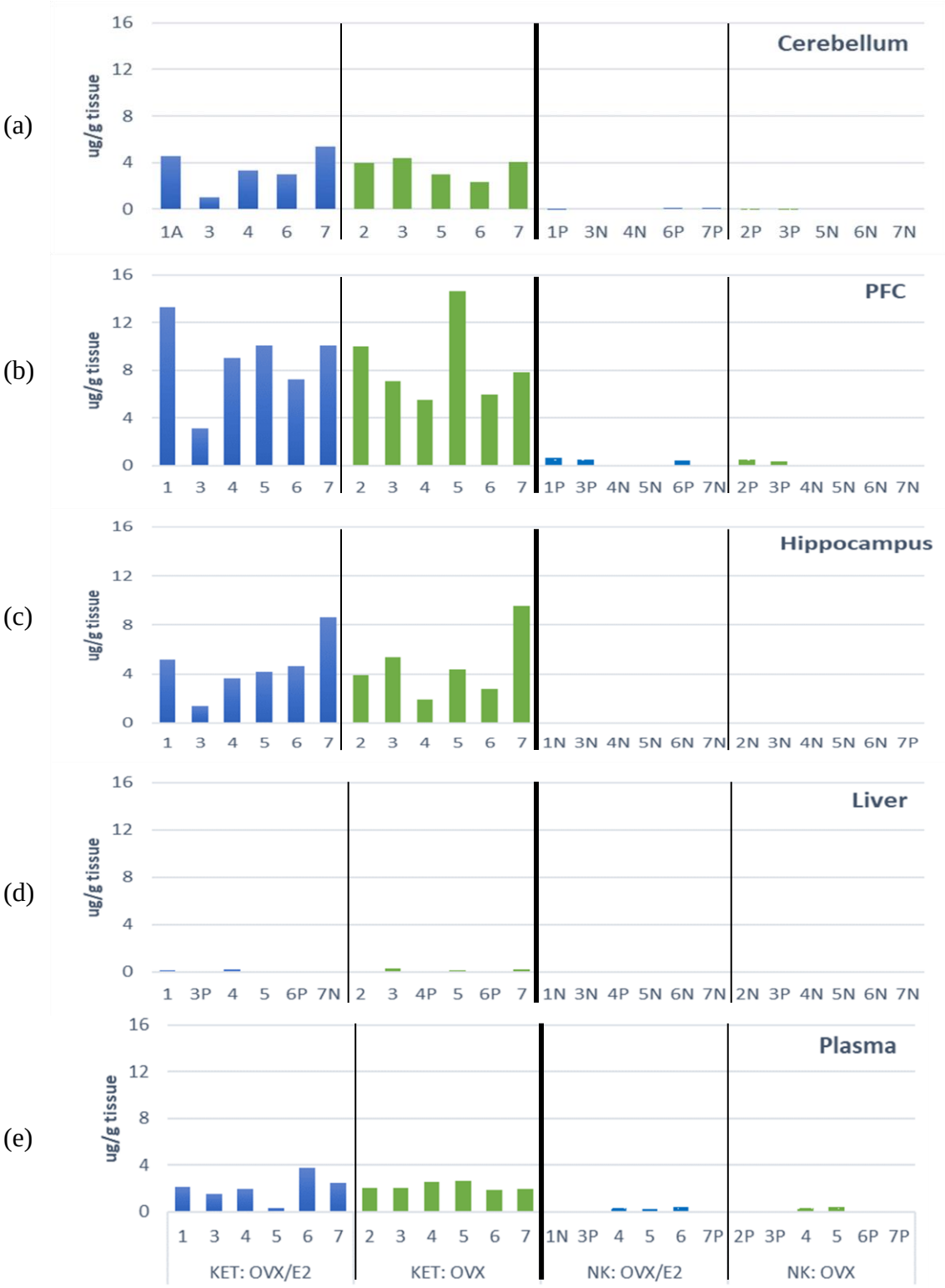

Figure 6. Concentrations of the ketamine (KET) and NK at time 0 minutes in the biological samples from the OVX and OVX/E2 rats: (a) Cerebellum, (b) PFC, (c), Hippocampus, (d) Liver, and (e) Plasma. The numbers correspond to the sample within that type. If the number is followed by "N", the analyte of interest was not detected. If followed by "P", the analyte was present but was below the LOQ. If followed by "A", the analyte was above the LOQ.

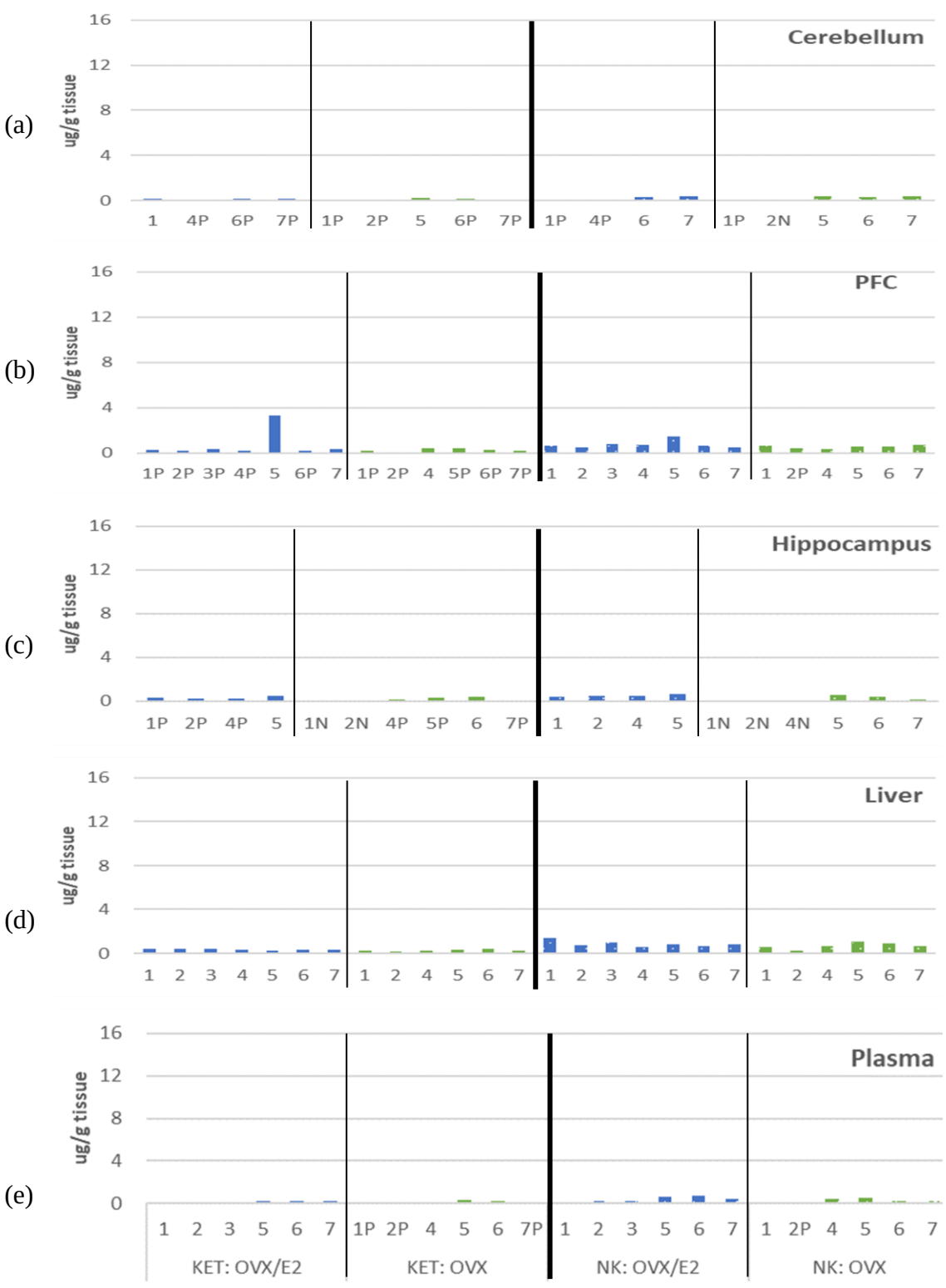

Figure 7. Concentrations of the ketamine (KET) and NK at time 30 minutes in the biological samples from the OVX and OVX/E2 rats: (a) Cerebellum, (b) PFC, (c), Hippocampus, (d) Liver, and (e) Plasma. The numbers correspond to the sample within that type. If the number is followed by "N", the analyte of interest was not detected in that sample. If followed by "P", the analyte was present but was below the LOQ.

Ketamine

In all the samples, except the liver tissue, the concentration of ketamine was greatest at time 0. The ketamine concentration was higher in the brain samples, particularly the PFC, than in the plasma and liver at time 0. The greatest ketamine concentrations in the PFC samples were 13.31 µg/g tissue in the OVX/E2 rats and 14.61 µg/g tissue in the OVX rats (Figure 8). By 30 minutes, ketamine was below the LOQ in all but one cerebellum, two PFC, and one hippocampus samples. It was greatest in one OVX/E2 rat, which had 3.31 µg/g tissue in the PFC.

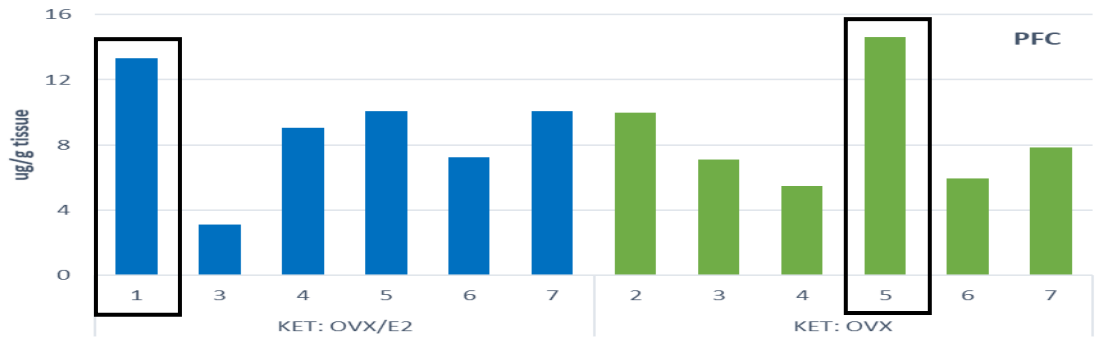

Figure 8. Ketamine (KET) concentrations (µg/g tissue) in the PFC of OVX and OVX/E2 rats at time 0 minutes. The black boxes indicate the highest concentration of ketamine in each type of rat.

Norketamine

At time 0, only the plasma samples had NK at detectable levels above the LOQ. The highest concentration of NK in the plasma was 0.38 µg/g tissue for the OVX/E2 rats and 0.39 µg/g tissue for the OVX rats (Figure 9). At 30 minutes, NK was greatest in the PFC and the liver. The highest concentration of NK in the PFC was 1.48 µg/g tissue for the OVX/E2 rats and 0.71 µg/g tissue for the OVX rats (Figure 10). In the liver, the greatest amounts of NK were 1.39 µg/g tissue for the OVX/E2 rats and 1.09 µg/g tissue for the OVX rats (Figure 11).

The negative control samples from the rats injected with saline solution instead of ketamine were negative for both ketamine and NK.

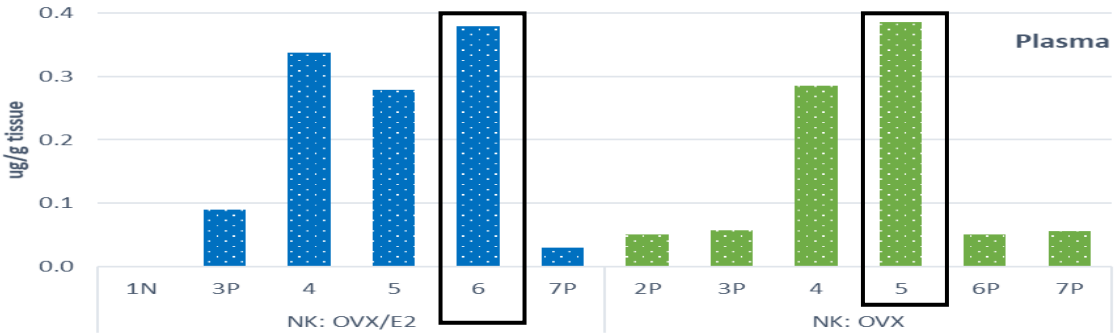

Figure 9. NK concentrations (µg/g tissue) in the plasma of OVX and OVX/E2 rats at time 0 minutes. The black boxes indicate the highest concentration of NK in each type of rat.

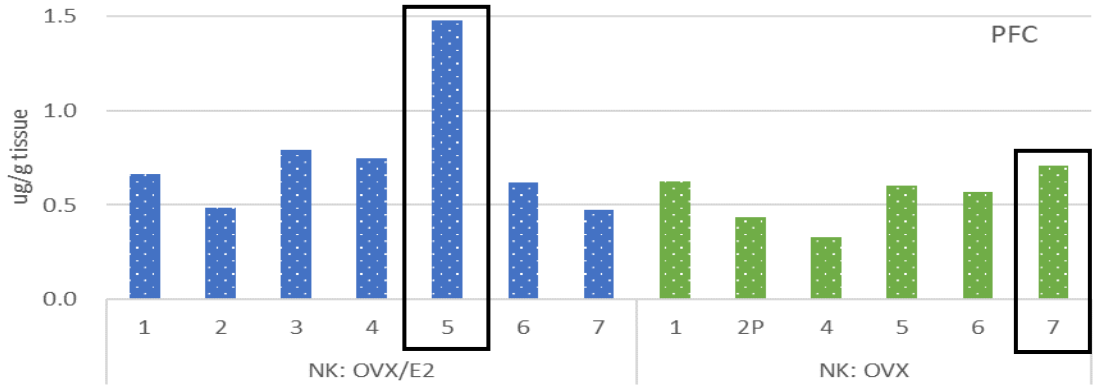

Figure 10. NK concentrations (µg/g tissue) in the PFC of OVX and OVX/E2 rats at time 30 minutes. The black boxes indicate the highest concentration of NK in each type of rat.

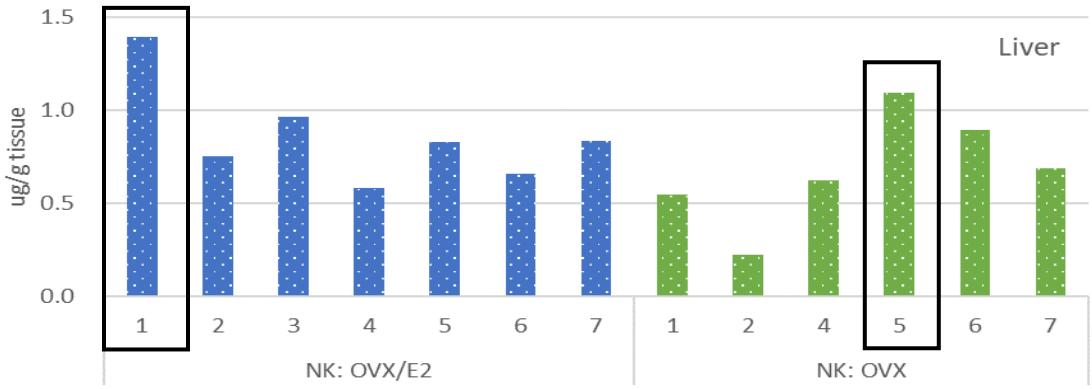

Figure 11. NK concentrations (µg/g tissue) in the liver of OVX and OVX/E2 rats at time 30 minutes. The black boxes indicate the highest concentration of NK in each type of rat.

DISCUSSION

Ketamine was at the greatest concentration in all tissues, except the liver, at time 0 and is essentially gone from the tissues by time 30 minutes. This shows that ketamine causes changes within the brain very quickly even though the antidepressant effects can take 2-24 hours to appear.[8]

The amount of ketamine was highest in the brain and specifically in the PFC. This result is consistent with the study conducted by Cohen et al.[30] who reported that ketamine accumulated in the brain, with a peak concentration at less than a minute. Because ketamine traveled to the brain so quickly, they proposed that there is almost no blood-brain barrier for this drug.

Even though at time 0, NK was below the LOQ in almost of the samples, it was still detected in the following samples: cerebellum 1.2, 4.1, 6.2, 11.2, and 13.2; PFC 1.2, 4.1, 5.2, 6.2, and 11.2; hippocampus 14.2; liver 6.2 and 7.2; and plasma 4.1, 5.2, 6.2, 12.2, 13.2, and 14.2. It was above the LOQ only in plasma samples 7.2, 8.1, 9.2, 10.2, and 11.2. This indicates that ketamine begins to metabolize very quickly. At time 30 minutes, the amount of NK was highest in the PFC and liver. The amount of NK directly correlates to the ketamine quickly accumulating in the PFC within minutes. Cohen et al.[30] reported that NK increased in concentration and was retained in the brain as well. It is not unexpected to see more NK in the liver at 30 minutes, because this tissue is a primary site for the metabolism of drugs by P450 cytochromes, the enzymes responsible for metabolizing ketamine into NK.[18]

Estrogen was given to the OVX/E2 rats in two injections given a day apart to simulate the proestrus stage of the estrus cycle. The ketamine injection was given 24 hours after the second estrogen injection. The gonadal hormones are highest during proestrus. Therefore, the likelihood of seeing a difference between the genders at this stage is greatest if estrogen is responsible for the differences in response to ketamine observed in both human subjects and rats.

There was not a noticeable difference in the amount of ketamine levels for the OVX and OVX/E2 female rat samples at time 0 or 30 minutes. The NK was below the LOQ for both sample sets at time 0 minutes. However, at time 30 minutes, the OVX/E2 rats had more NK present than the OVX rats, overall. In the PFC and the liver, the OVX/E2 rats had slightly higher concentrations of NK than the OVX rats. The ranges of NK concentrations in the PFC were 0.17-0.75 µg/g tissue in the OVX/E2 rats and 0.03-0.54 µg/g tissue in the OVX rats. In the liver, the amount of NK was 0.58-1.39 µg/g tissue in the OVX/E2 rats and 0.11-1.09 µg/g tissue in the OVX rats. In the hippocampus, the NK concentrations in all four OVX/E2 samples were above the LOQ, but the NK was above the LOQ in only three of the seven OVX samples and was not detected in three of those samples. Finally, all seven of the OVX/E2 plasma samples had levels of NK that were above the LOQ. The NK was above the LOQ in only five of the seven OVX plasma samples. The Kolmogorov-Smirnov test (KS test) was run for the PFC samples at 30 minutes to confirm normal distribution. Then a two-tailed Student's t-test for independent samples was conducted. The test showed that at the 90% confidence level, there is not a significant difference between the OVX and OVX/E2 NK levels in the PFC at time 30 minutes and that these results warrant further study.

The main difference between the OVX and OVX/E2 rat was the amount of NK at time 30 minutes. Since the OVX/E2 rats overall had more NK in the PFC and liver and more NK detected in the hippocampus and plasma, this may suggest that estrogen is slightly increasing the metabolism of ketamine to NK. As noted by Carrier et al.[27], female rats responded to the lower ketamine doses (2.5 mg/kg). Future work might include administering lower doses to determine if the differences in NK concentration are enhanced in the OVX/E2 rats. Further, other metabolites, especially HNK, may need to be detected and quantitated to better understand the full effect of estrogen on the metabolism of ketamine.

CONCLUSIONS

The main purpose of this research was to study the role the gonadal hormones play in the metabolism of the fast-acting antidepressant ketamine in rats. The levels of ketamine and its primary metabolite NK were quantitated in five different tissues collected from OVX and OVX/E2 female rats at 0 and 30 minutes after injection.

The results of this study indicate that ketamine accumulates in the brain, primarily in the PFC, within minutes and is cleared from the tissues by 30 minutes. The NK was either below the LOQ or was not detected in any of the tissues at time 0 but could be detected in tissues 30 minutes post injections. The most NK was quantified in the PFC and liver at 30 minutes.

The primary difference between the OVX and OVX/E2 rats was the amount of NK at 30 minutes. Overall, the OVX/E2 rats had more NK in the PFC and liver and could be detected, but not quantified, in the hippocampus and plasma. By 30 minutes, the ketamine had time to metabolize, so differences in the amount of NK in the rats likely are due to the presence of the estrogen. These results suggest that the estrogen may cause more of the ketamine to be metabolized or increase rate of metabolism. However, these results and the study by Erb[29] suggest that the gender differences in response may not be due to estrogen altering the metabolism of ketamine as much as it is causing the drug to interact differently with the NMDA receptor in the brain. Erb did not see a significant distinction between male and OVX female rats. In this research, the only difference

between the OVX and OVX/E2 rats was the concentration of NK at 30 minutes, but statistical tests showed that this variation was not significantly different. Therefore, males and females may respond to ketamine differently because of the changes in the brain caused by estrogen rather than because of any effect of estrogen on ketamine metabolism.

This study could be expanded by quantitating other metabolites, specifically HNK, in OVX and OVX/E2 rats to better determine if males and females metabolize ketamine differently. Further, the rats can be given multiple doses to evaluate how the antidepressant effects and concentrations of the different compounds change over time.

The toxicological results from this graduate research project will be correlated with the behavioral observation studies conducted by the McMahon group to better understand how ketamine affects the genders. If the use of ketamine as an antidepressant becomes more wide-spread, it will be important to understand how males and females respond to this drug since this will affect the dosage given to patients and how to treat each gender in the long term.

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APPENDIX A

GC/MS METHOD PARAMETERS

Method Parameters	**Experiment Time:**	30 minutes
	Inlet Mode:	Split-Ratio 5:1
	Column Mode:	Constant Flow (1.0 mL/min)
	Injection Volume:	2 µL
	Initial Oven Temperature:	175°C for 10 Minutes
	Oven Program:	20°/min until temp. reaches 250°C; Hold for 16.25 min.
	Injector Temperature:	250ºC
	Detector:	Mass Selective in Vacuum
	SIM Parameters:	**NK**: Monitor ions 131, 166, 170, and 195 from 5-9.40 minutes (9.80 minutes after 12/4/17) **Ketamine and Ketamine-d_4**: Monitor Ions 138, 152, 180, and 184 from 9.40 min. (9.80 min. after 12/4/17) to end **Dwell time of 50 msec for each ion**
Column and Gas Information	**Column Information:**	Agilent HP-5MS: 30 m x 250 µm x 0.25 µm
	Carrier Gas:	Helium (UHP)
	Total Flow Rate:	1 mL/min.

Table A1. SIM GC/MS Parameters for Ketamine and NK.

APPENDIX B

MASSES OF SAMPLES

CEREBELLUM			
Animal	**Mass (g)**	**Ket (ng/µL)**	**NK (ng/µL)**
1.1	0.317	0.45	BQ
1.2	0.340	AQ	BQ
2.1	0.459	ND	ND
2.2	0.320	BQ	BQ
3.1	0.282	ND	ND
3.2	0.299	bad run	bad run
4.1	0.287	11.48	BQ
4.2	0.301	BQ	ND
5.1	0.316	bad run	bad run
5.2	0.347	3.40	ND
6.1	0.351	BQ	BQ
6.2	0.264	11.47	BQ
7.1	0.261	BQ	BQ
7.2	0.267	8.89	ND
8.1	0.306	bad run	bad run
8.2	0.305	bad run	bad run
9.1	0.289	bad run	bad run
9.2	0.264	bad run	bad run
10.1	0.258	0.56	0.97
10.2	0.261	7.67	ND
11.1	0.294	BQ	0.95
11.2	0.249	7.33	BQ
12.1	0.274	BQ	0.87
12.2	0.282	6.56	ND
13.1	0.298	BQ	1.13
13.2	0.257	13.78	BQ
14.1	0.153	BQ	0.60
14.2	0.337	13.61	ND
15.1	0.350	ND	ND
15.2	0.265	ND	ND

Table B1. Masses (g) of the 30 cerebellum samples and the concentrations of ketamine (Ket) and NK, both in ng/µL, in each sample. The "**ND**" denotes that the analyte was not detected in the sample, "**BQ**" indicates that the analyte was present but was below the LOQ, and "**AQ**" means the analyte was above the LOQ. If the data was not able to be obtained, the sample is marked "**bad run".** Animals 15.1 and 15.2 were the negative control rats.

PFC			
Animal	**Mass (g)**	**Ket (ng/µL)**	**NK (ng/µL)**
1.1	0.085	BQ	0.56
1.2	0.068	9.00	BQ
2.1	0.098	BQ	ND
2.2	0.097	BQ	0.60
3.1	0.121	BQ	ND
3.2	0.116	BQ	0.56
4.1	0.092	9.21	BQ
4.2	0.112	BQ	BQ
5.1	0.078	BQ	0.62
5.2	0.102	3.20	BQ
6.1	0.105	BQ	BQ
6.2	0.122	8.63	BQ
7.1	0.086	BQ	0.64
7.2	0.092	8.36	ND
8.1	0.098	5.41	ND
8.2	0.160	0.70	0.53
9.1	0.144	4.76	2.13
9.2	0.100	10.08	ND
10.1	0.103	BQ	0.62
10.2	0.068	9.92	ND
11.1	0.093	BQ	0.58
11.2	0.096	6.92	BQ
12.1	0.100	BQ	0.57
12.2	0.094	5.57	ND
13.1	0.144	0.52	0.68
13.2	0.132	13.30	ND
14.1	0.081	BQ	0.58
14.2	0.168	13.20	ND
15.1	0.097	ND	ND
15.2	0.119	ND	ND

Table B2. Masses (g) of the 30 PFC samples and the concentrations of ketamine (Ket) and NK, both in ng/µL, in each sample. The "**ND**" denotes that the analyte was not detected in the sample, and the "**BQ**" indicates that the analyte was present but was below the LOQ. Animals 15.1 and 15.2 were the negative control rats.

HIPPOCAMPUS			
Animal	**Mass (g)**	**Ket (ng/µL)**	**NK (ng/µL)**
1.1	0.127	BQ	0.55
1.2	0.131	6.74	ND
2.1	0.109	ND	ND
2.2	0.112	ND	ND
3.1	0.141	ND	ND
3.2	0.131	BQ	0.61
4.1	0.137	5.36	ND
4.2	0.100	0.10	ND
5.1	0.116	bad run	bad run
5.2	0.120	1.65	ND
6.1	0.105	BQ	BQ
6.2	0.130	6.93	ND
7.1	0.117	BQ	0.55
7.2	0.111	4.07	ND
8.1	0.127	2.41	ND
8.2	0.129	BQ	ND
9.1	0.128	0.61	0.84
9.2	0.111	4.64	ND
10.1	0.109	BQ	0.64
10.2	0.113	4.93	ND
11.1	0.125	bad run	bad run
11.2	0.132	6.15	ND
12.1	0.148	0.59	0.61
12.2	0.121	3.35	ND
13.1	0.087	bad run	bad run
13.2	0.110	9.47	ND
14.1	0.119	BQ	BQ
14.2	0.137	13.12	BQ
15.1	0.115	ND	ND
15.2	0.126	ND	ND

Table B3. Masses (g) of the 30 hippocampus samples and the concentrations of ketamine (Ket) and NK, both in ng/µL, in each sample. The "**ND**" denotes that the analyte was not detected in the sample, and the "**BQ**" indicates that the analyte was present but was below the LOQ. If the data was not able to be obtained, the sample is marked "**bad run".** Animals 15.1 and 15.2 were the negative control rats.

LIVER			
Animal	**Mass (g)**	**Ket (ng/µL)**	**NK (ng/µL)**
1.1	0.874	3.37	12.18
1.2	0.518	0.63	ND
2.1	1.005	ND	ND
2.2	1.028	2.15	5.64
3.1	0.888	ND	ND
3.2	1.027	3.96	7.76
4.1	0.822	0.53	ND
4.2	0.944	1.46	2.14
5.1	0.755	2.93	7.28
5.2	0.756	BQ	ND
6.1	1.248	0.97	1.42
6.2	1.146	3.54	BQ
7.1	1.258	3.81	7.32
7.2	0.755	1.77	BQ
8.1	0.837	BQ	ND
8.2	0.765	1.82	4.76
9.1	0.992	2.78	8.22
9.2	1.156	0.65	ND
10.1	0.699	2.10	7.64
10.2	1.090	1.18	ND
11.1	0.725	2.55	4.77
11.2	0.831	BQ	ND
12.1	0.651	2.56	5.81
12.2	0.542	BQ	ND
13.1	0.811	2.81	6.76
13.2	1.024	ND	ND
14.1	0.774	2.19	5.35
14.2	0.727	1.39	ND
15.1	0.531	ND	ND
15.2	0.784	ND	ND

Table B4. Masses (g) of the 30 liver samples and the concentrations of ketamine (Ket) and NK, both in ng/µL, in each sample. The "**ND**" denotes that the analyte was not detected in the sample, and the "**BQ**" indicates that the analyte was present but was below the LOQ. If the data was not able to be obtained, the sample is marked "**bad run".** Animals 15.1 and 15.2 were the negative control rats.

PLASMA			
Animal	**Mass (g)**	**Ket (ng/µL)**	**NK (ng/µL)**
1.1	0.505	0.58	0.83
1.2	0.505	10.65	ND
2.1	0.504	ND	ND
2.2	0.503	BQ	0.64
3.1	0.497	BQ	BQ
3.2	0.501	0.72	0.93
4.1	0.498	10.35	BQ
4.2	0.502	BQ	BQ
5.1	0.494	0.64	1.18
5.2	0.501	7.51	BQ
6.1	0.501	BQ	BQ
6.2	0.497	10.17	BQ
7.1	0.486	bad run	bad run
7.2	0.493	9.57	1.66
8.1	0.490	12.53	1.40
8.2	0.487	0.82	1.82
9.1	0.491	1.05	2.85
9.2	0.492	1.48	1.37
10.1	0.492	1.33	2.64
10.2	0.495	13.25	1.91
11.1	0.495	1.04	3.69
11.2	0.492	18.62	1.87
12.1	0.510	0.98	1.32
12.2	0.479	9.08	BQ
13.1	0.482	0.83	1.95
13.2	0.494	12.12	BQ
14.1	0.485	BQ	1.19
14.2	0.481	9.37	BQ
15.1	0.497	ND	ND
15.2	0.494	ND	ND

Table B5. Masses (g) of the 30 plasma samples and the concentrations of ketamine (Ket) and NK, both in ng/µL, in each sample. The "**ND**" denotes that the analyte was not detected in the sample, and the "**BQ**" indicates that the analyte was present but was below the LOQ. If the data was not able to be obtained, the sample is marked "**bad run".** Animals 15.1 and 15.2 were the negative control rats.

APPENDIX C

REFERENCE CODE FOR ANIMAL NUMBERS

TIME 0			TIME 30		
Type	Rat #	Graph #	Type	Rat #	Graph #
OVX/E2	1.2	1	OVX/E2	1.1	1
	3.1*	2		3.2	2
	5.2	3		5.1	3
	7.2	4		7.1	4
	9.2	5		9.1	5
	11.2	6		11.1	6
	13.2	7		13.1	7
OVX	2.1	1	OVX	2.2	1
	4.1	2		4.2	2
	6.2	3		**6.1***	3
	8.1	4		8.2	4
	10.2	5		10.1	5
	12.2	6		12.1	6
	14.2	7		14.1	7

Table C1. Reference code that correlates the rat number from the behavioral study with the sample number on the graphs in the toxicological portion of the study. Rats 3.1 and 6.1 were reported as receiving injections that potentially missed the vein.