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Title: Ketamine produces lasting disruptions in encoding of sensory stimuli

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Running Title: Ketamine and evoked potentials

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Abstract

The current study analyzed the acute, chronic and lasting effects of ketamine administration in four inbred mouse strains (C3H/HeHsd, C57BL/6Hsd, FVB/Hsd, DBA/2Hsd) to evaluate vulnerability to ketamine as a drug of abuse and as a model of schizophrenia. Serum half-life of ketamine was similar between all strains (approximately 13 min). Also, the ratio of brain to serum ketamine levels was 3 to 1. Examination of multiple phases of auditory processing using auditory evoked potentials (AEP) following acute ketamine (0, 5, 20 mg/kg) treatment revealed C3H/HeHsd mice to be most vulnerable to ketamine-induced alterations in AEP whereas FVB/Hsd mice exhibited the least electrophysiological sensitivity to ketamine. Overall, the pre-cortical P1 evoked potential component increased in amplitude and latency while the cortically-generated N1 and P2 components decreased in amplitude and latency following acute ketamine across all strains. Brain catecholamine analyses indicated that ketamine decreased hippocampus epinephrine levels in C3H/HeHsd but elevated hippocampus epinephrine levels in FVB/Hsd, suggesting one potential mechanism for AEP vulnerability to ketamine. Based on results of the acute study, the immediate and lasting effects of chronic low dose ketamine on AEP were examined among C3H/HeHsd (sensitive) and FVB/Hsd (insensitive) mice. We observed a decrement of the N1 amplitude that persisted at least one week after the last exposure to ketamine across both strains. This lasting deficit in information processing occurred in the absence of acute changes among the FVB/Hsd mice. Implications for both ketamine abuse and N-Methyl D-Aspartate hypofunction models of schizophrenia are discussed.

Introduction

N-Methyl D-Aspartate receptor antagonists such as ketamine are frequently abused by adolescents, college students, military and medical personnel (Gahlinger, 2004; Morgan et al., 2004). Although these drugs produce hallucinations and delusions as well as exacerbating pre-existing psychosis, several studies have argued that the psychotic and dissociative features are less prominent than the lasting cognitive disruptions (Malhotra et al., 1997; Morgan et al., 2004). Indeed, the nature of these lasting cognitive deficits remains unclear and few studies have distinguished the effects of ketamine on the multiple phases of information processing. The current study employs auditory evoked potentials (AEP) to examine the acute, chronic and lasting effects of ketamine on various phases of sensory encoding in mice. In so doing, we will further elucidate the precise phases of information processing during which N-Methyl D-Aspartate antagonists affect sensory processing.

The physiological and cognitive effects of N-Methyl D-Aspartate -antagonist drugs of abuse are also relevant to models of schizophrenia. Acute administration of ketamine produces behavioral, cognitive and physiological AEP changes consistent with schizophrenia (Malhotra et al., 1997; Newcomer et al., 1999; Umbricht et al., 2000; Micallef et al., 2002). Several investigators have proposed that reduced function at N-Methyl D-Aspartate receptors may precede overt manifestations of schizophrenia (Newcomer et al., 1999; Greene, 2001). It has been difficult to test such hypotheses in humans due to the inability to identify schizophrenia probands prior to their first-break episode (McConaghy, 2000). Alternatively, animal studies can measure acute, chronic

and lasting effects of N-Methyl D-Aspartate antagonists to evaluate both the potential risks of substance abuse and as models for understanding the neurobiology of schizophrenia.

Sensory encoding and information processing have been measured using the P1, N1 and P2 components of AEPs in humans and animals. The P1 reflects pre-cortical processing whereas the N1 and P2 are cortically-generated and can be modulated by directing the subject's attention during the auditory task (Picton et al., 1974; Gallinat et al., 2002). Preliminary studies in humans suggest that these components may have different sensitivities to the effects of acute N-Methyl D-Aspartate receptor blockade. For example, one study found that ketamine did not change gating of the human P1, but did not evaluate amplitude (van Berckel et al., 1998). Others found increased N1 amplitude with ketamine without alteration of the P2 (Umbricht et al., 2000). Initial studies in rodents suggest that the mouse P1 increases in amplitude following acute ketamine, whereas the mouse and rat P2 decrease in amplitude following acute ketamine exposure (de Bruin et al., 1999; Connolly et al., 2004). Although such studies suggest different sensitivities to acute N-Methyl D-Aspartate antagonists among various phases of auditory processing, little is known about the lasting effects of ketamine on such measures.

The current report utilizes AEPs as physiological measures to elucidate immediate and lasting effects of acute and chronic N-Methyl D-Aspartate receptor blockade on multiple phases of auditory processing. The acute exposure studies utilize 4 inbred

mouse strains to determine genetic vulnerability to ketamine as a predictor of chronic and lasting effects. Subsequent studies to assess the immediate and lasting effects of chronic exposure were then performed using one vulnerable and one resistant inbred strain. The inclusion of such natural genetic variation was intended to provide a foundation for future studies directed at addressing genetic susceptibility to N-Methyl D-Aspartate antagonists in humans.

Methods

Animals: A total of 260 mice were used in this study. Male C57BL/6Hsd, FVB/Hsd, C3H/HeHsd and DBA/2Hsd were obtained at 8 weeks of age from Harlan (Indianapolis, IN). All testing was conducted between 10-13 weeks of age. Mice were housed 4-5/cage in a light and temperature-controlled Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility. Water and standard rodent chow were available ad lib. Experiments were conducted at the University of Pennsylvania during the light phase between the hours of 0900 and 1300. Mice were acclimated to the housing facility for at least 1 week prior to all procedures. All protocols were performed in accordance with University Laboratory Animal Resources guidelines and were approved by the Institutional Animal Care and Use Committee.

Surgery: Animals underwent stereotaxic implantation of electrode assemblies (PlasticsOne Inc., Roanoke, VA) for non-anesthetized recording of auditory evoked potentials. The surgical procedure has been previously reported (Connolly et al., 2003; Siegel et al., 2003; Connolly et al., 2004; Maxwell et al., 2004a; Maxwell et al., 2004b). Animals were anesthetized with isoflurane. Unipolar recording electrodes were placed unilaterally in the CA3 hippocampal region, (1.4 mm posterior, 2.65 mm lateral and 2.75 mm deep relative to bregma) and referenced to the ispilateral frontal sinus to reflect whole brain electrical activity. The electrode pedestal was secured to the skull using dental cement and super glue. Electrode placement was verified to be in the target region using the Perl's iron reaction (LaBossiere and Glickstein, 1976).

AEP Treatment Groups

Acute Study: One hundred twenty eight mice were used in the AEP portion of this study. In the acute ketamine experiment, 32 mice (8 per strain, 4 strains) were used in each of the following conditions: vehicle (0 mg/kg), 5 mg/kg ketamine or 20 mg/kg intraperitoneal ketamine. These animals were tested two weeks after electrode surgery.

Chronic Study: The chronic exposure portion of this study utilized an additional thirty-two mice to model the effects of repeated ketamine abuse. FVB/Hsd and C3H/HeHsd mice received daily injections of saline (8 FVB/Hsd and 8 C3H/HeHsd) or 5 mg/kg ketamine (8 FVB/Hsd and 8 C3H/HeHsd) for two weeks. These animals were tested for the effects of chronic ketamine after two weeks of exposure and again for the lasting effects of ketamine seven days later.

Recording: The recording session for the AEP experiments consisted of a baseline, drug naive trial followed by an i.p. injection of either ketamine or vehicle. The recording parameters for the chronic experiments were identical to that of the acute with recording following the 14th daily injection. Drug exposure trials were recorded five minutes after the injection. Stimuli were generated by Micro1401 hardware and Spike 5 software (Cambridge Electronic Design, Cambridge, England) and were delivered through speakers attached to the cage top. A series of 50 white noise clicks (10 milliseconds in duration) were presented with a 9 second interstimulus interval at 85 dB compared to background of 70 dB. Waveforms were filtered between 1 and 500 Hz, baseline corrected at stimulus onset and individual sweeps were rejected for movement

artifact based on a criterion of two times the root mean squared amplitude per mouse. Average waves were created from 50 ms pre-stimulus to 200 ms post stimulus (Figure 1). All recordings were performed within the animal's home cage, which was placed in a Faraday cage 15 minutes prior to stimulus onset.

Analysis: The amplitude and latency of the P1 (most positive deflection between 10 and 30 ms), N1 (most negative deflection between 20 and 70 ms) and P2 (most positive deflection between 60 and 200 ms) were analyzed. A general liner models Analysis of Variance was used to determine main effects and interactions of ketamine and strain, where drug condition and strain were designated independent variables. The next portion of this study evaluates influences of strain and ketamine on habituation of each auditory component during the acute ketamine administration study. The average of the responses to the first 25 stimuli was compared to the average of the responses to the last 25 stimuli (habituation variable) for each component using a repeated measures Analysis of Variance with strain and ketamine designated independent variables and habituation designated the repeated measure. Significant interactions were followed up with Fisher Least Significant Difference post hoc analyses.
Alpha levels were set at $p < 0.05$ for all analyses.

Serum Half-life: This experiment determined the serum half-life of ketamine in four mouse strains. Eighty additional mice (4 per strain at each of 5 time points) were injected with 100 mg/kg intraperitoneal ketamine and sacrificed at 5 (n=16), 10 (n=16), 20 (n=16), 30 (n=16) and 60 (n=16) minutes after injection. The dose of 100 mg/kg was

used to facilitate a detectable level at 60 minutes after injection. Serum was pooled to allow for amplification one hour after injection which produces a mean value per strain, per time point. The HPLC protocol for ketamine extraction in serum is adapted from the phencyclidine extraction protocol provided by Waters Corporation (Milford, MA) for use with Breeze software. The wavelength for ketamine was 210 nm with a retention time of 7.4 minutes. The exponential decay equation: $x(t) = x(0)e^{at}$ where t (time) and x(0) (initial value or x-intercept) are used to calculate the unknown decay constant (a). Once the decay constant (a) is determined, the half life equation is $t_{1/2} = \ln 0.5/a$.

Serum and Brain Concentration: Thirty-two mice (8 mice per strain) were injected i.p. with 20 mg/kg acute ketamine to evaluate serum for ketamine and norketamine, a ketamine metabolite, fifteen minutes after injection to determine serum concentration at the time consistent with AEP testing. The ketamine extraction protocol is described above. Norketamine was evaluated using the same parameters with a retention time of 4.6 minutes. An Analysis of Variance followed by Fisher Least Significant Difference post hoc analyses were performed to determine strain differences in metabolism of ketamine and norketamine. Twelve additional FVB/Hsd mice were sacrificed after fifteen minutes of exposure to 0, 5, 20 or 100 mg/kg ketamine. Serum and brain levels were assayed for ketamine and norketamine to determine the correlation between serum and brain ketamine levels at time of AEP testing.

Regional Catecholamine levels in Brain: The effects of ketamine on brain catecholamine levels in vulnerable (C3H/HeHsd) and resistant (FVB/Hsd) strains were

evaluated in order to investigate a potential neurochemical mechanism by which ketamine alters evoked potentials. Eight FVB/Hsd and eight C3H/HeHsd mice were treated with 0 or 20 mg/kg ketamine to evaluate dopamine, norepinephrine and epinephrine levels in hypothalamus and hippocampus. Animals were sacrificed using rapid decapitation fifteen minutes after injection. The brains were removed, regionally dissected and immediately placed in ice cold 0.1M $HClO_4$ spiked with an internal standard of 3,4-dihydroxybenzylamine. Samples were frozen at -80 degrees C until catecholamines were assayed by HPLC with electrochemical detection. Briefly, samples were sonicated and centrifuged with aliquots of the supernatants added to sodium phosphate buffer (pH 6.1), 1.5M Tris/EDTA buffer (pH 8.6) and acid-washed alumina. The alumina was washed in water and the catecholamines were eluted off the alumina with 0.1M $HClO_4$. This extract from each sample was manually injected into the HPLC. The HPLC system consisted of pumps (Varian ProStar) and a C_{18} reverse-phase column (150 mm x 4.6, 5 µ) located in a temperature controlled housing (27 ^{0}C) with an electrochemical detector (Varian Star 9080). The system was operated at a flow rate of 1.0 ml/min with the detector potential set at 0.75 V versus an Ag-AgCl reference electrode. The mobile phase buffer consisted of 0.33 M citrate, 0.67 M phosphate (pH 4.5) with sodium octyl sulfate (1.2 mM) and 12% methanol. The signal from the detector was recorded by a PC computer (Dell Optiplex) fitted with an ADC controller card using Varian Star Chromatography software (Star Chromatography, version 5).

Results

Pharmacokinetic Analyses

Serum Half-life: The decay constant (a) was found to be -0.053. This yielded a serum half-life of thirteen minutes for ketamine across 80 mice and 4 strains. The half-life of ketamine in each strain is as follows: C57BL/6Hsd (a = -0.055) 12.7 minutes, DBA/2Hsd (a = -0.050) 14.0 minutes, FVB/Hsd (a = -0.051) 13.7 minutes and C3H/HeHsd (a = -0.060) 11.6 minutes (Figure 2A).

Serum Concentration: Main effects of strain were found for both ketamine ($F(3, 28) = 5.14$, $p = 0.006$) and norketamine ($F(3, 28) = 4.34$, $p = 0.012$) levels. DBA/2Hsd mice had higher serum ketamine concentration than FVB/Hsd, C3H/HeHsd and C57BL/6Hsd. In addition, C57BL/6Hsd mice have significantly lower norketamine concentrations compared to all strains. We also evaluated the relationship between serum concentration of ketamine and whole brain ketamine levels within a subset of FVB/Hsd mice. Our results indicate a 3.33:1 ratio of brain to serum ketamine concentration (μg/ml serum and ng/mg brain) and a 1.43:1 ratio of brain to serum norketamine levels (ketamine correlation: $r = 0.996$, $p<0.001$; norketamine correlation: $r = 0.993$, $p<0.001$) (Figure 2 B, C & D).

Acute AEP

Trends: Main effects of ketamine and interactions between ketamine and strain indicate that ketamine influenced amplitudes and latencies of the P1, N1 and P2. Across all four strains, the main effects of ketamine displayed the following trends: 1) Ketamine

increased the P1 amplitude and lengthened the P1 latency, 2) Ketamine decreased N1 amplitude and shortened N1 latency and 3) Ketamine did not change P2 amplitude but shortened P2 latency. The strain by ketamine interactions described below suggests that there are also strain-dependent vulnerabilities to ketamine (Table 1 & 2).

Strain-specific findings:

C3H/HeHsd**:** P1 amplitude and latency did not change with ketamine in this strain. The N1 amplitude dose-dependently decreased with ketamine administration whereas the latency remained unchanged. The P2 amplitude did not change with ketamine but the latency dose-dependently decreased (Figure 3 & 4).

FVB/Hsd: P1, N1 and P2 amplitudes and latencies did not change with ketamine exposure in this strain.

C57BL/6Hsd: P1 amplitude increased with ketamine exposure whereas the latency remained unchanged. The N1 amplitude was unchanged with ketamine exposure while the N1 latency decreased. The P2 amplitude was unchanged while the P2 latency decreased at 20 mg/kg.

DBA/2Hsd: The 5 mg/kg dose of ketamine increased the P1 amplitude without changes in latency. The N1 amplitude increased with both doses of ketamine while latency was unchanged. The P2 amplitude was unchanged with ketamine whereas the P2 latency decreased at both doses.

Regional Catecholamine analyses: This analysis evaluated dopamine, norepinephrine and epinephrine levels in hippocampus and hypothalamus in two mouse strains defined to be resistant (FVB/Hsd) and sensitive (C3H/HeHsd) based on acute AEP data. We found that FVB/Hsd mice have more dopamine in hypothalamus compared to C3H/HeHsd irrespective of ketamine treatment. Also, hippocampus epinephrine levels were reduced with ketamine in C3H/HeHsd mice ($p = 0.035$) relative to a saline injection in C3H/HeHsd mice but elevated with ketamine in FVB/Hsd mice compared to the FVB/Hsd saline group ($p = 0.019$), (Table 3, Figure 5).

The next series of experiments were performed to evaluate the chronic and lasting effects of ketamine exposure. We selected two strains that differ for the degree of vulnerability to acute ketamine for this analysis. We chose to use C3H/HeHsd and FVB/Hsd mice for this comparison based on their differential effects on the N1 amplitude. Additionally, these strains were selected because they had similar serum levels of both ketamine and norketamine, facilitating a comparison of CNS mechanisms of vulnerability without peripheral metabolic variation.

Chronic and Lasting AEP:

In this design, AEP were recorded from C3H/HeHsd and FVB/Hsd mice after receiving daily ketamine (5 mg/kg) or saline i.p. injections for 14 days. The dose of 5 mg/kg of ketamine was chosen based on the acute data in which C3H/HeHsd mice show decreased N1 amplitude at this dose, while FVB/Hsd did not. Additionally, to determine

lasting effects of ketamine, AEP were recorded on day 21 (7 days after last injection of ketamine or saline).

P1:

Chronic and Lasting Effects: There were no main effects of ketamine or interactions with strain on the amplitude or latency of the P1 after the final daily injection or seven days later (Table 4).

N1:

Chronic Effects: There were no main effects of ketamine or interactions with strain on the amplitude or latency of the N1 after the final daily injection on day 14.

Lasting Effects: A main effect of ketamine demonstrated that ketamine treatment produced a reduction of N1 amplitude on day 21 relative to animals that received matched saline injections (Figure 6). There were no lasting effects on the latency of the N1. Also, there were no interactions between strain and ketamine on either measure of this component.

P2:

Chronic and Lasting Effects: There were no main effects of ketamine or interactions with strain on the amplitude or latency of the P2 after the final daily injection or seven days later.

Habituation of AEP:

P1: There were no main effects of habituation or interactions with strain and ketamine on the amplitude of the P1 (Table 5).

N1: An interaction between strain and habituation indicated that C57BL/6Hsd mice showed significantly higher N1 amplitude in response to the last 25 stimuli relative to the first 25. No other strains displayed significant differences between the first and last 25 stimuli presented (Table 6). A second interaction between habituation and ketamine demonstrated that the 20 mg/kg dose of ketamine significantly decreased the amplitude of response to the first 25 stimuli relative to the last 25 stimuli, suggesting that the inhibitory effects of ketamine were attenuated during progression of the task (Table 7).

P2: There were no main effects of habituation or interactions with strain and ketamine on the amplitude of the P2 (Table 5).

Discussion

The current study demonstrates both acute and lasting alterations in sensory encoding of auditory stimuli following ketamine exposure. Acute effects were characterized by increased P1 amplitude with decreased N1 amplitude and decreased P2 latency. These studies also defined differential vulnerability to ketamine among 4 inbred mouse strains, suggesting that genetic background influences the physiologic response to this class of drugs of abuse. Among these differences, C3H/HeHsd and DBA/2Hsd mice demonstrate opposing reactions for N1 amplitude following acute ketamine whereas C57BL/6Hsd and FVB/Hsd mice were insensitive to its effects. Also, pharmacokinetic data allowed for evaluation of peripheral metabolic factors that may have influenced this differential sensitivity. These data indicated that DBA/2Hsd mice had higher serum ketamine and C57BL/6Hsd mice had lower serum norketamine than other strains, limiting the interpretation of differences among the AEP findings in these two inbred strains. However, C3H/HeHsd and FVB/Hsd mice have similar levels of both ketamine and its main metabolite, indicating that differential vulnerability was more likely due to central neuronal factors. This hypothesis is further supported by the modulation of hippocampal epinephrine levels with ketamine in C3H/HeHsd and FVB/Hsd mice.

These data suggest that epinephrine is a central factor that could influence strain specific ketamine vulnerability through alterations in hippocampal catecholamine concentrations. Specifically, ketamine decreases hippocampal epinephrine levels in C3H/HeHsd mice which have decreased AEP amplitudes, demonstrating a vulnerability to N-Methyl D-Aspartate receptor antagonism. Ketamine administration increases

epinephrine levels in the ketamine resistant strain (FVB/Hsd) which show no alterations in AEP. One rodent study showed systemic epinephrine increased the N1-P2 AEP amplitude, suggesting that epinephrine may modulate late latency AEP (Berntson et al., 2003). Similarly, human studies have proposed that the hippocampus plays a role in sensory gating of late-latency AEP (Grunwald et al., 2003). To our knowledge, this is the first report of differential AEP sensitivities to acute ketamine in mice that coincide with changes in hippocampal epinephrine concentrations. There are several limitations to the interpretation of this finding. Since other brain regions were not evaluated, a generalized increase in epinephrine concentrations throughout the brain may occur in FVB/Hsd with ketamine and an overall decrease may occur in C3H/HeHsd mice. Similarly, dopamine and norepinephrine levels may be altered with ketamine treatment in other brain regions not examined in this study. Also, our data reflect a snap shot of epinephrine levels at a single time point and therefore can not distinguish changes in synthesis from changes in release and/or turnover. Future studies will evaluate catecholamine levels in multiple brain regions of animals treated with acute and chronic ketamine to further explore catecholamine modulation of AEP.

Based on the acute AEP and pharmacokinetic findings, C3H/HeHsd and FVB/Hsd strains were selected as vulnerable and resistant strains, respectively, to evaluate chronic and lasting alterations following 14 days of repeated low dose exposure. Interestingly, neither strain displayed alterations in AEP measures directly following the final daily exposure, suggesting adaptation among the previously vulnerable C3H/HeHsd group. Equally intriguing, both groups then demonstrated lasting decrement of the N1

amplitude following 7 days without ketamine exposure, indicating sensitivity to ketamine in FVB/Hsd mice over time. As we demonstrate, the serum half-life for this drug is approximately 13 minutes in mouse, strongly suggesting that no ketamine remains in the animal at the final time point, which equals 775 half-lives after final administration. Thus, genetic heterogeneity to the physiologic effects of ketamine does not appear to protect against the lasting alterations in the neural substrates of sensory encoding of the N1 AEP.

Acute ketamine exposure in both human and animal literature is also commonly used to model schizophrenia-like deficits in information processing. Multiple studies have shown that decreased N1 amplitude is a reproducible physiological characteristic of schizophrenia (Lifshitz et al., 1987; Boutros et al., 1999; Boutros et al., 2004a; Boutros et al., 2004b). Additionally, recent data from the clinical Schizophrenia Research Center affiliated with our group demonstrates both decreased N1 amplitude and reduced P2 latency among people with schizophrenia (Turetsky et al., in review). Therefore, AEP alterations secondary to reduced N-Methyl D-Aspartate -mediated glutamate transmission following acute low dose ketamine are consistent with the profile seen in schizophrenia. However, these deficits were not found following repeated exposure in mice, suggesting that adaptive changes within the brains of C3H/HeHsd mice may have normalized N-Methyl D-Aspartate receptor function in the presence of low dose ketamine. Such adaptive changes appear to have remained 7 days after the last exposure, resulting in lasting deficits in sensory encoding. These observations (decreased N1 amplitude and decreased P2 latency) are consistent with the idea that N-Methyl D-Aspartate

hypofunction may be involved in both the cognitive deficits of schizophrenia and among people who abuse ketamine as a recreational drug. Alternatively, the P2 latency shift may not represent an independent phenomenon if it results from a decrease in preceding N1 amplitude. While this is a concern in most AEP studies, some investigators have made an effort to describe the P2 as an independent component (Crowley and Colrain, 2004).

Secondary analyses of the acute data in this study evaluated the effects of strain and ketamine on habituation to auditory stimuli. Our results indicate that the N1 response in C57BL/6Hsd mice increased over the course of the trial, while other strains did not. Thus, these data indicate that auditory AEPs do not habituate over the course of a single test session. Also, the amplitude of the N1 was lower for the first 25 responses relative to the second 25 following the 20 mg/kg dose of ketamine across all strains, suggesting a loss of inhibition over the course of the task. The loss of ketamine-induced inhibition of the N40 is unlikely to reflect clearance of the active drug because the effect was only manifest on a single component and all testing occurred within one serum half-life for ketamine.

There are a number of limitations to this study. Both C3H/HeHsd and DBA/2Hsd mice display retinal degeneration which leads to blindness in adult animals (Farber and Lolley, 1973; Schuettauf et al., 2004). This spontaneous visual impairment could promote cortical compensation from the auditory system and may influence the physiological response to auditory stimuli. However, our data indicate that C3H/HeHsd

and DBA/2Hsd mice have opposite evoked responses at baseline and during ketamine treatment suggesting that compensation for visual deficits may not be a major factor influencing the results found in this study. Also, while previous studies propose that the P1 reflects thalamic and early cortical activity while the N1 and P2 reflect higher order processing involving auditory cortex and association cortices, the specific generators of these components are difficult to isolate in mice. Although the direct analogy between human and mouse AEP components are unclear, this study and others provide further support that the human P1, N1 and P2 are analogous to the mouse P1, N1 and P2, respectively (Connolly et al., 2003; Siegel et al., 2003; Connolly et al., 2004; Maxwell et al., 2004a; Maxwell et al., 2004b; Umbricht et al., 2004; Siegel et al., 2005). One additionally clarification in this study regards the method of chronic ketamine exposure. The chronic portion of this study utilized repeated daily injections to ketamine rather than continuous exposure from pumps or implants. This paradigm was selected to model chronic ketamine abuse, during which people self-administer acute doses repeatedly over time. However, this may differ from proposed mechanisms of chronic, continuous N-Methyl D-Aspartate hypofunction during the schizophrenia prodrome, perhaps limiting generalizability to that disease model.

In summary, the current study indicates that inbred mouse strains differ in their physiological AEP response to acute N-Methyl D-Aspartate receptor blockade. Our results also support the hypothesis that ketamine acts primarily to reduce cortical processing while having the opposite effect on pre-cortical processing. Of note, neither the sensitive nor resistant strains showed alterations following chronic exposure,

suggesting the presence of adaptive, compensatory mechanisms in N-Methyl D-Aspartate-mediated transmission. Similarly, both strains demonstrated lasting deficits in sensory encoding, suggesting that long-term adaptations to chronic N-Methyl D-Aspartate hypofunction occur even in the absence of acute effects.

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Footnotes

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Legends for Figures

Figure 1 displays the grand average waveform for control (black), 5 mg/kg (light gray) and 20 mg/kg (dark gray) acute ketamine in FVB/Hsd, C3H/HeHsd, C57BL/6Hsd and DBA/2Hsd mice. The P1, N1 and P2 are labeled. The arrows indicate ketamine-induced latency shift of the P2.

Figure 2 displays the results for pharmacokinetic experiments. Figure **2A** demonstrates the serum half-life of ketamine across four strains ($t_{½}$ = 13 minutes). Figure **2B** displays the mean ± sem for serum concentrations of ketamine and norketamine (ketamine metabolite). Fisher Least Significant Difference post hoc analyses indicate C57BL/6Hsd differ from DBA/2Hsd ($p < 0.001$) for ketamine concentrations; and DBA/2Hsd are marginally different from C3H/HeHsd ($p = 0.056$) and FVB/Hsd ($p = 0.041$) (MS = 75068, df = 28). Analyses for norketamine concentrations indicate that C57BL/6Hsd differ from C3H/HeHsd, DBA/2Hsd and FVB/Hsd ($p = 0.007$, $p = 0.009$, $p = 0.004$) (MS = 82917, df = 289). Figure **2C** demonstrates the 3.33**:**1 linear relationship between ketamine concentration in brain (ng/mg) and serum (μg/ml). Figure **2D** demonstrates the 1.42**:**1 linear relationship between norketamine concentration in brain and serum. Points in figures **2C** and **2D** represent data from 5, 20 and 100 mg/kg ketamine (n = 3/dose).

Figure 3 shows the mean + sem for the amplitude of the P1, N1 and P2 components for each strain and dose of ketamine in the acute study. Note, * indicates a significant difference ($p<0.05$) from the other inbred strains at each dose; and τ indicates significant

difference ($p<0.05$) from the control condition in the same strain using Fisher Least Significant Difference post hoc (P1: MS = 1401, df = 84; N1 MS = 2146, df = 84; P2 no interaction)

Figure 4 shows the mean + sem for the latency of the P1, N1 and P2 components for each strain and dose of acute ketamine. Note, * indicates a significant difference ($p<0.05$) from the other inbred strains at each dose; and τ indicates significant difference ($p<0.05$) from the control condition in the same strain using Fisher Least Significant Difference post hoc (P1: no interaction; N1 MS = 0.00006, df = 84; P2 MS = 0.0089, df = 84). Only C57BL/6Hsd mice show decreased latency of N1, and all strains except FVB/Hsd display decreased latency of P2 following ketamine.

Figure 5 displays catecholamine levels in hippocampus and hypothalamus in FVB/Hsd and C3H/HeHsd mice following either saline or ketamine injections. The levels (mean + sem) for (A) epinephrine and (B) norepinephrine in hippocampus are shown for FVB/Hsd and C3H/HeHsd mice treated with saline and ketamine. Note that ketamine induces a reduction in hippocampal epinephrine in C3H/HeHsd and an elevation in FVB/Hsd mice. The levels (mean + sem) for (C) norepinephrine and (D) dopamine in hypothalamus are shown for FVB/Hsd and C3H/HeHsd mice treated with saline and ketamine. Dopamine levels were undetectable in hippocampus and epinephrine levels were undetectable in hypothalamus. * indicates $p<0.05$.

Figure 6 demonstrates the mean + sem for the lasting effects of chronic low dose ketamine (5 mg/kg/day for 14 days) or saline in C3H/HeHsd and FVB/Hsd mice. There was no significant two-way interaction between ketamine and strain for the N1, suggesting that chronic ketamine affected this measure to the same extent in both strains. The main effect of ketamine resulted in decreased N1 amplitude across both strains.

Tables

Table 1: Amplitude and latency of AEP components following acute ketamine

	Variable	P1	N1	P2
Amplitude	Strain	**F(3, 84) = 4.9, p =0.003**	**F(3, 84) = 4.3, p =0.007**	**F(3, 84) = 4.8, P =0.004**
	Ketamine	**F(2, 84) = 4.5, p =0.014**	**F(2, 84) = 5.2, p =0.007**	F(2, 84) = 0.7, P =0.481
	Strain by Ketamine	**F(6, 84) = 2.5, p =0.025**	**F(6, 84) = 4.9, p <0.001**	F(6, 84) = 0.5, P =0.794
Latency	Strain	**F(3, 84) = 4.0, p =0.010**	**F(3, 84) = 15.8, p <0.001**	**F(3, 84) = 17.9, P <0.001**
	Ketamine	**F(2, 84) = 4.4, p =0.015**	F(2, 84) = 2.9, p =0.057	**F(2, 84) = 17.1, P <0.001**
	Strain by Ketamine	F(6, 84) = 2.0, p =0.067	**F(6, 84) = 2.9, p =0.011**	**F(6, 84) = 3.0, P =0.009**

Table 1 provides the statistical values for strain, ketamine and strain by ketamine interactions for the amplitude and latency of the P1, N1 and P2 AEP components. Significant effects (α level set at p. <0.05) of acute ketamine are highlighted in bold.

Table 2: Schematic representation of trends for amplitude and latency of the P1, N1 and P2 following acute ketamine

	P1		N1		P2	
Strain	Amp	Lat	Amp	Lat	Amp	Lat
Across All	↑	↑	↓	↓	—	↓
C3H	—	—	↓	—	—	↓
C57	↑	—	—	↓	—	↓
DBA	↑	—	↑	—	—	↓
FVB	—	—	—	—	—	—

Table 2 describes the acute trends and strain by ketamine interactions for the P1, N1 and P2.

Table 3: Hippocampus and hypothalamus catecholamine levels following acute ketamine

	Hippocampus		Hypothalamus	
Variable	Norepinephrine	Epinephrine	Dopamine	Norepinephrine
Strain	F(1,12) = 0.35, p = 0.567	F(1,12) = 0.18, p = 0.683	**F(1,12)= 5.44, p = 0.038**	F(1,12) = 0.71, p = 0.418
Ketamine	F(1,12) = 0.23, p = 0.642	F(1,12) = 0.05, p = 0.833	F(1,12) = 3.51, p = 0.085	F(1,12) = 0.11, p = 0.751
Strain by ketamine	F(1,12) = 0.36, p = 0.561	**F(1,12) = 6.23, p = 0.028**	F(1,12) = 1.79, p = 0.205	F(1,12) = 0.15, p = 0.703

Table 3 shows the statistical values for regional catecholamine levels following 20 mg/kg ketamine in C3H/HeHsd and FVB/Hsd mice. Dopamine levels in hippocampus and epinephrine levels in hypothalamus were undetectable in these samples.

Table 4: Amplitude and latency of AEP components following chronic ketamine

	Variable	P1 Chronic	P1 Lasting	N1 Chronic	N1 Lasting	P2 Chronic	P2 Lasting
Amplitude	Strain	F(1,28) = 1.3, p=0.269	F(1,28) = 1.4, p=0.246	**F(1,28) = 12, p<0.001**	**F(1,28) = 4.2, p=0.050**	F(1,28) = 0.9, p=0.341	F(1,28) = 1.5, p=0.225
	Ketamine	F(1,28) = 0.9, p=0.340	F(1,28) < 0.1, p=0.899	F(1,28) = 0.2, p=0.692	**F(1,28) = 5.5, p=0.026**	F(1,28) = <0.1, p=0.950	F(1,28) = 2.1, p=0.155
	Strain by Ketamine	F(1,28) = 0.1, p=0.745	F(1,28) = 2.2, p=0.149	F(1,28) = 1.6, p=0.223	F(1,28) = 1.2, p=0.291	F(1,28) = 0.9, p=0.345	F(1,28) < 0.1, p=0.890
Latency	Strain	F(1,28) = 3.1, p=0.086	F(1,28) = 0.8, p=0.387	**F(1,28) = 9.7, p<0.001**	F(1,28) = 0.8, p=0.375	F(1,28) = 0.3, p=0.586	F(1,28) = 4.1, p=0.054
	Ketamine	F(1,28) = 0.7, p=0.413	F(1,28) = 0.1, p=0.748	F(1,28) = 0.6, p=0.451	F(1,28) = 1.2, p=0.276	F(1,28) = 4.0, p=0.056	F(1,28) = 3.1, p=0.091
	Strain by Ketamine	F(1,28) = 0.5, p=0.507	F(1,28) = 4.0, p=0.057	F(1,28) < 0.1, p=0.914	F(1,28) < 0.1, p=0.950	F(1,28) < 0.1, p=0.818	F(1,28) = 1.0, p=0.325

Table 4 displays the statistical values for the chronic and lasting ketamine experiments.

Significant effects are highlighted in bold.

Table 5 Habituation analyses of the P1, N1 and P2

	P1	N1	P2
Habituation	$F(1,84) < 0.01$, $p = 0.993$	$F(1,84) = 1.14$, $p = 0.289$	$F(1,84) = 0.02$, $p = 0.892$
Strain by Habituation	$F(3,84) = 1.94$, $p = 0.130$	**$F(3,84) = 2.99$, $p = 0.036$**	$F(3,84) = 0.30$, $p = 0.823$
Ketamine by Habituation	$F(2,84) = 0.53$, $p = 0.588$	**$F(2,84) = 3.54$, $p = 0.034$**	$F(2,84) = 1.02$, $p = 0.364$
Strain by Ketamine by Habituation	$F(6,84) = 0.71$, $p = 0.646$	$F(6,84) = 1.50$, $p = 0.190$	$F(6,84) = 0.49$, $p = 0.817$

Table 5 demonstrates the statistical values for habituation analyses of the P1, N1 and P2 AEP components.

Table 6: Interaction of habituation and strain

		Habituation by Strain	
Strain	Habituation	Mean ± S.E.M.	P value
C3H	1st Half	-117.603 ± 12.10618	p = 0.057
	2nd Half	-93.775 ± 12.06173	
FVB	1st Half	-134.017 ± 12.10618	p = 0.470
	2nd Half	-142.987 ± 12.06173	
DBA	1st Half	-91.618 ± 12.10618	p = 0.202
	2nd Half	-107.517 ± 12.06173	
C57	1st Half	-69.128 ± 12.10618	**p = 0.043**
	2nd Half	-94.466 ± 12.06173	

Table 6 demonstrates the statistical values for the Fisher Least Significant Difference post-hoc analyses of strain and habituation.

Table 7: Interaction of habituation and ketamine

		Habituation by Ketamine	
Ketamine	Habituation	Mean ± S.E.M.	P value
Vehicle	1st Half	-114.798 ± 10.48	p = 0.467
	2nd Half	-106.970 ± 10.45	
5 mg/kg	1st Half	-118.006 ± 10.48	p = 0.854
	2nd Half	-116.032 ± 10.45	
20 mg/kg	1st Half	-76.470 ± 10.48	**p = 0.007**
	2nd Half	-106.057 ± 10.45	

Table 7 demonstrates the statistical values for the Fisher Least Significant Difference post-hoc analyses of ketamine and habituation.

Figure 1

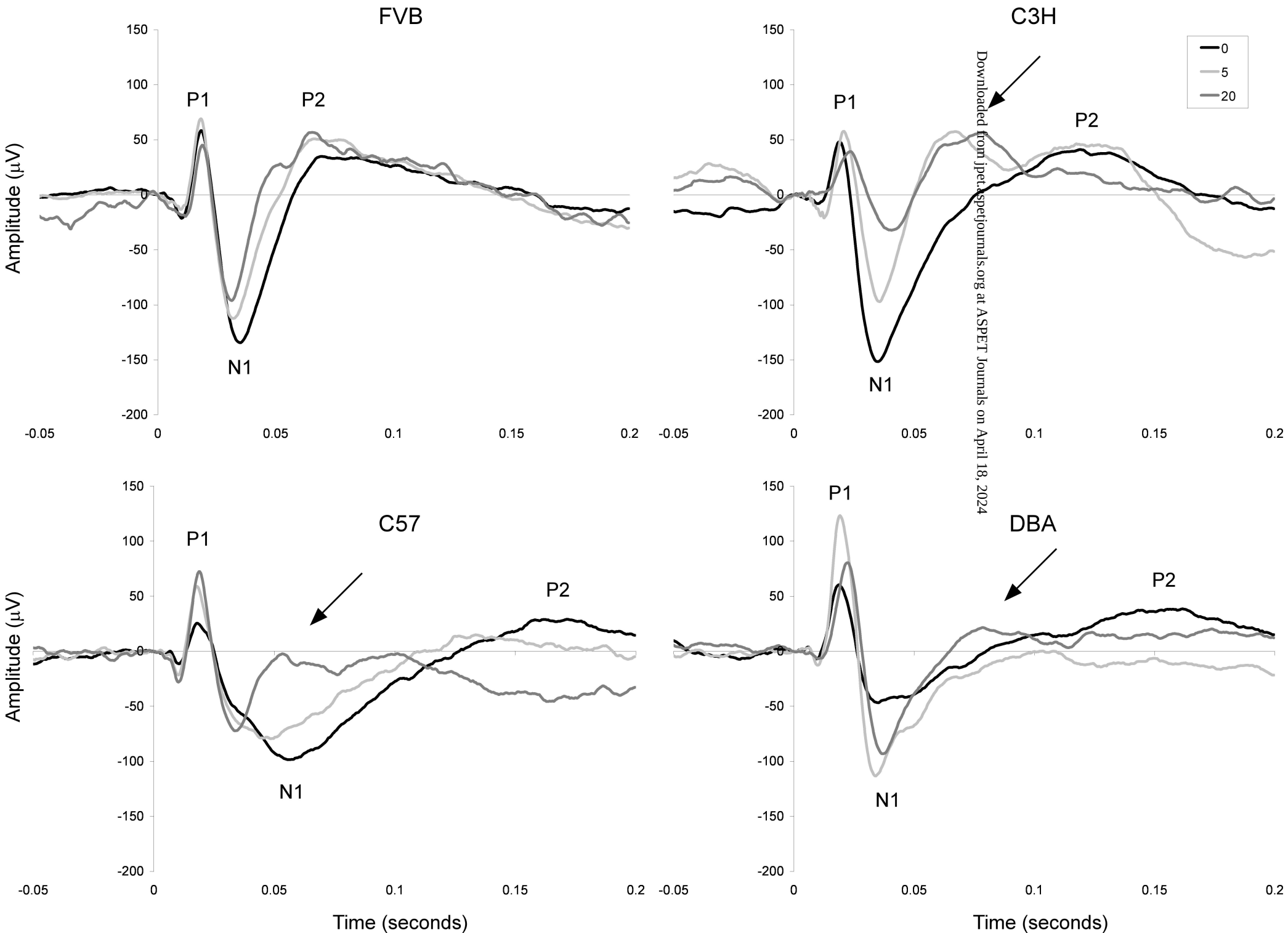

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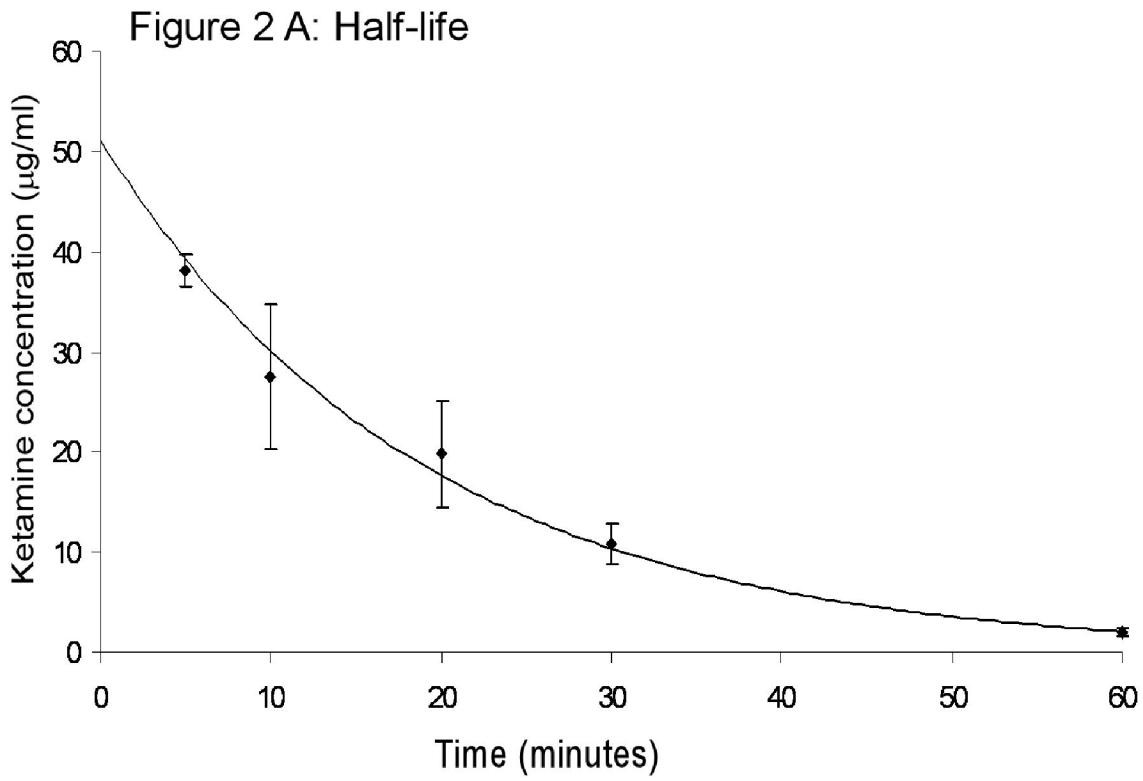
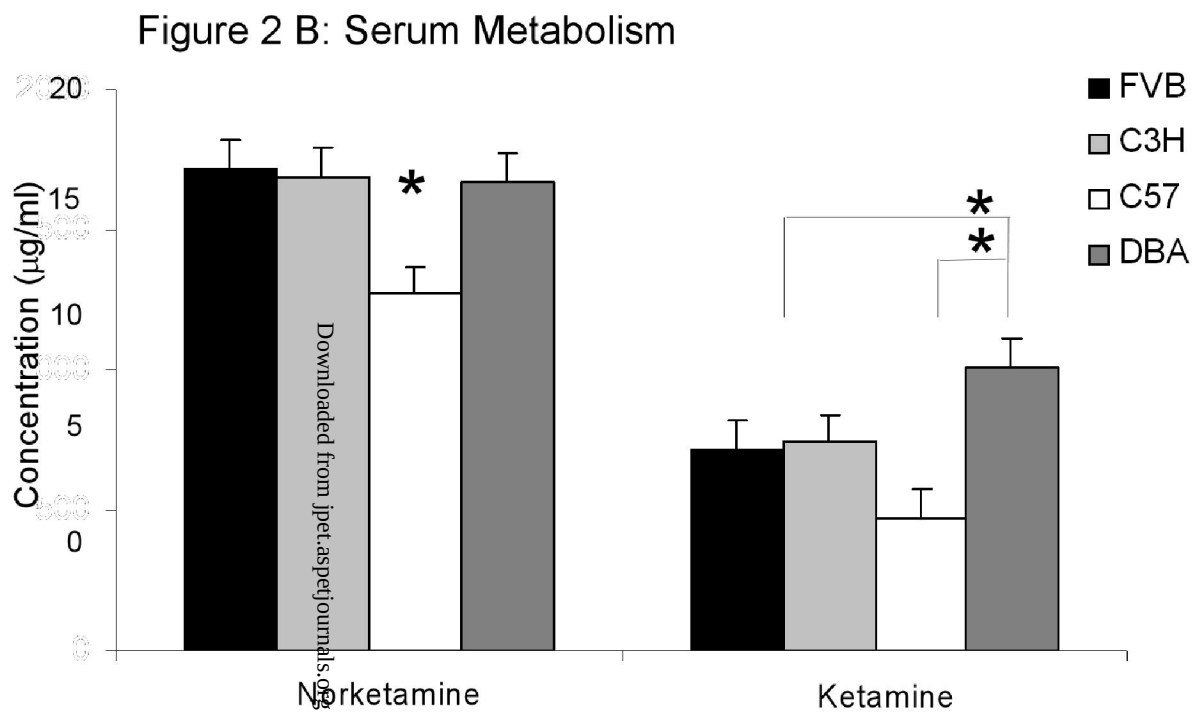
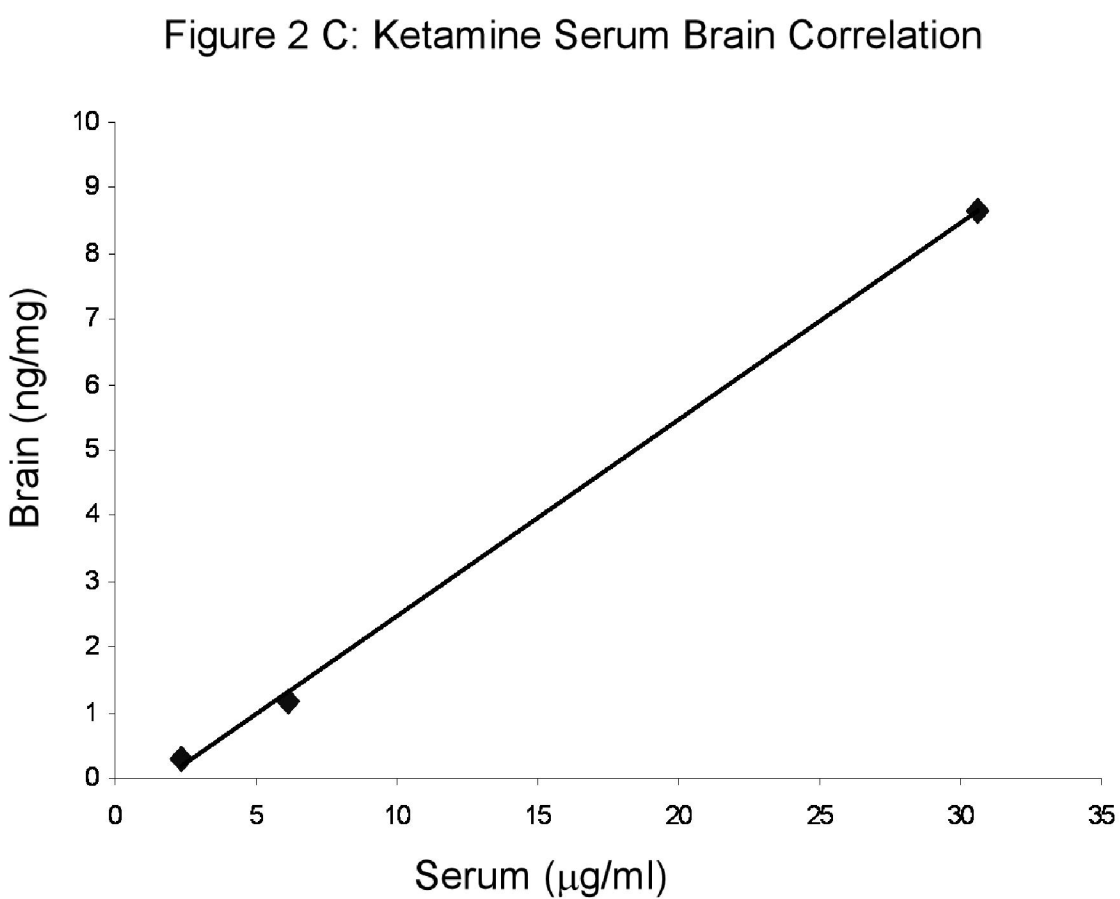
Figure 2







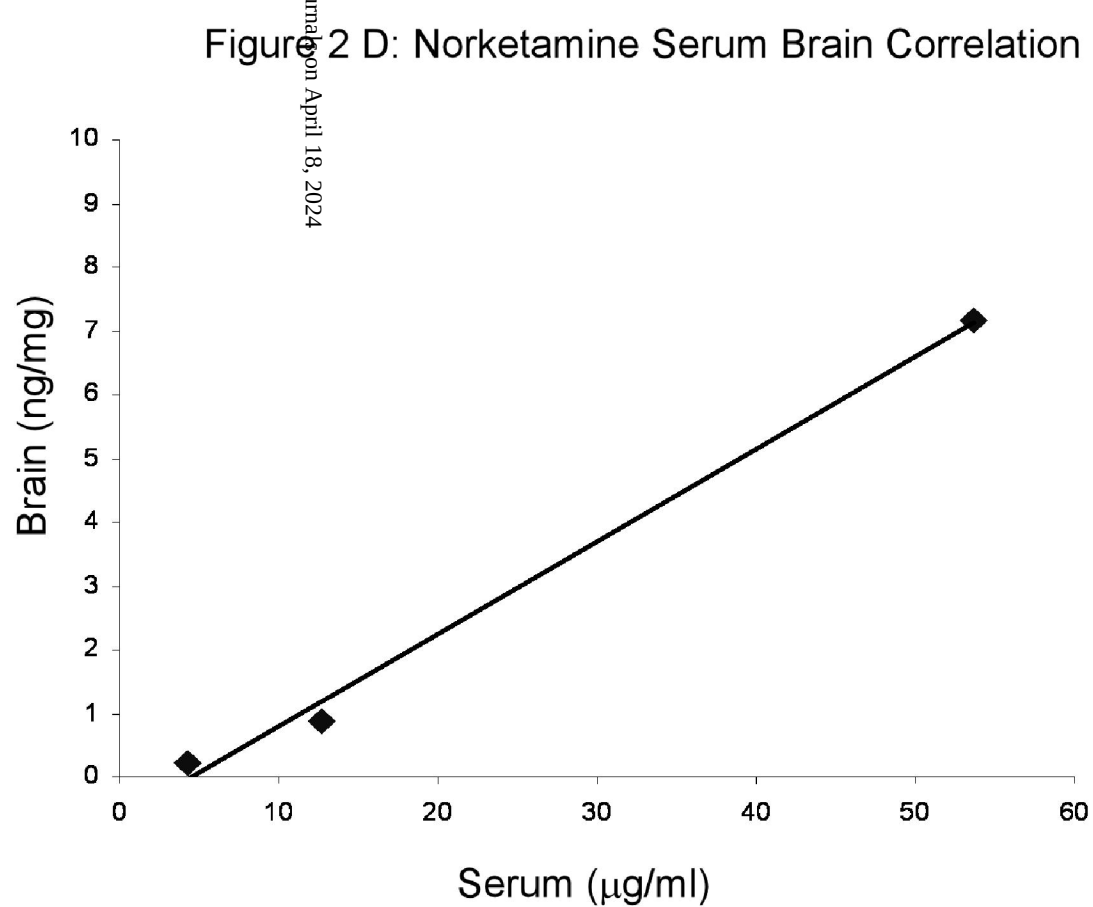


Figure 3

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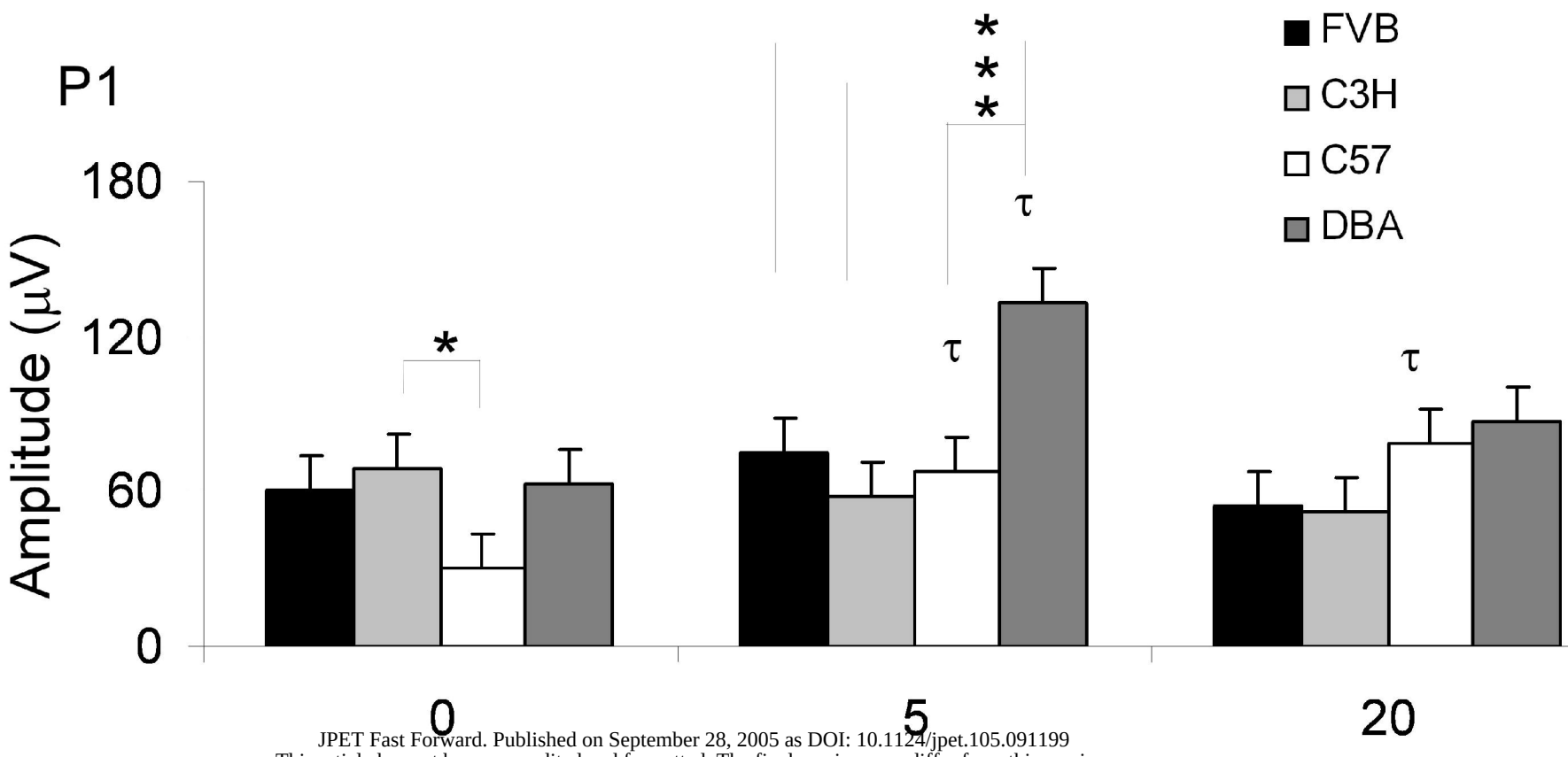

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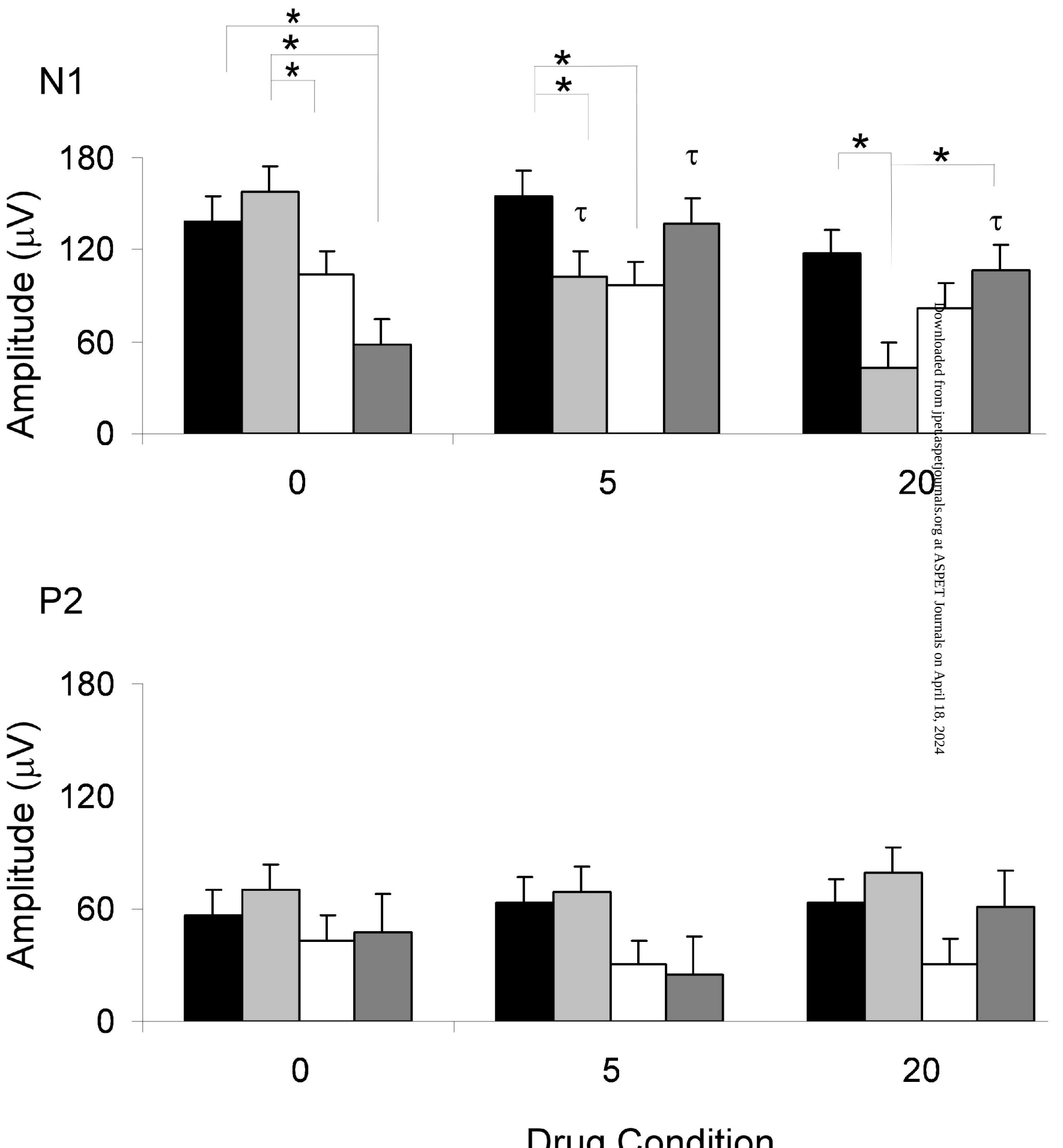

Figure 4

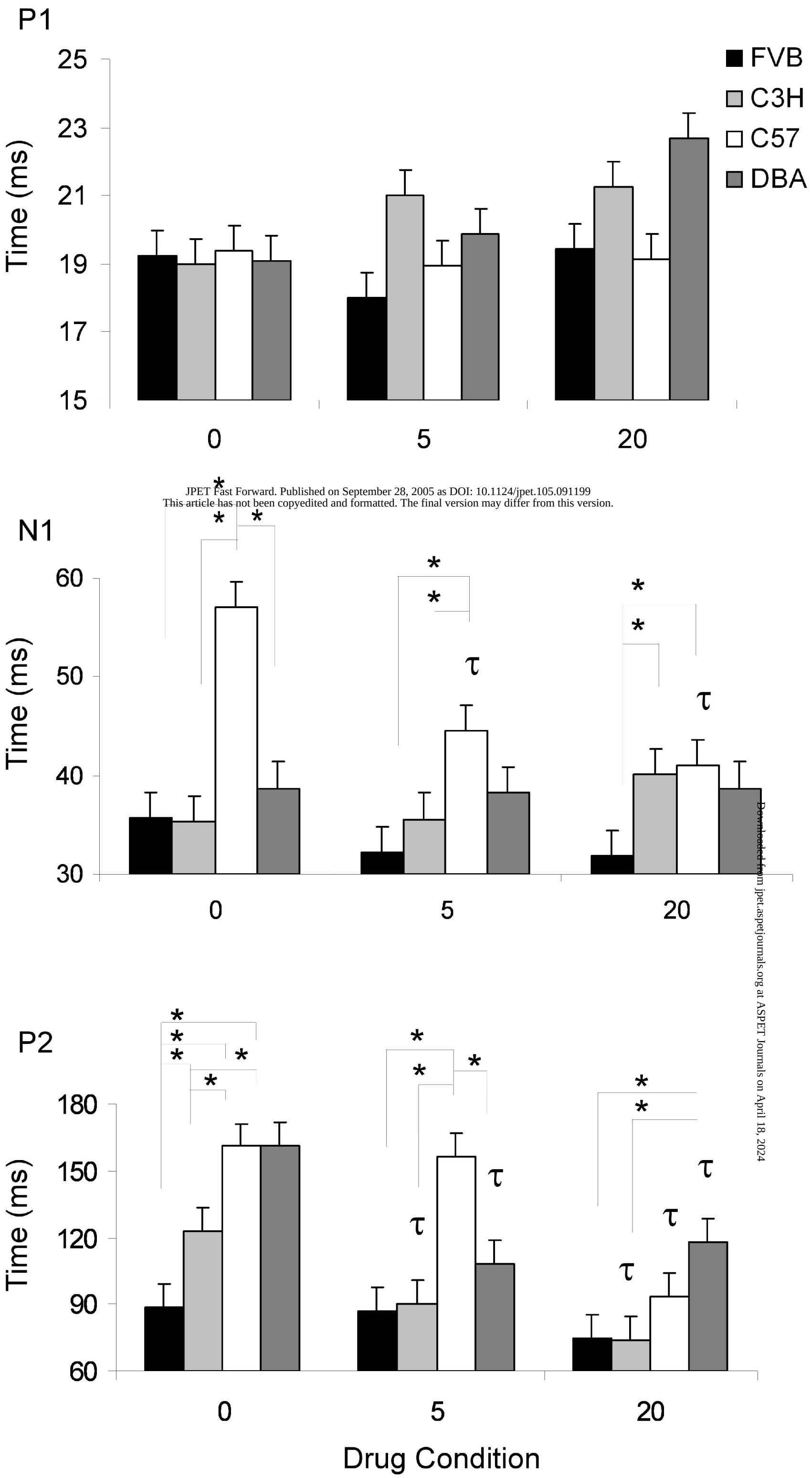

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Figure 5

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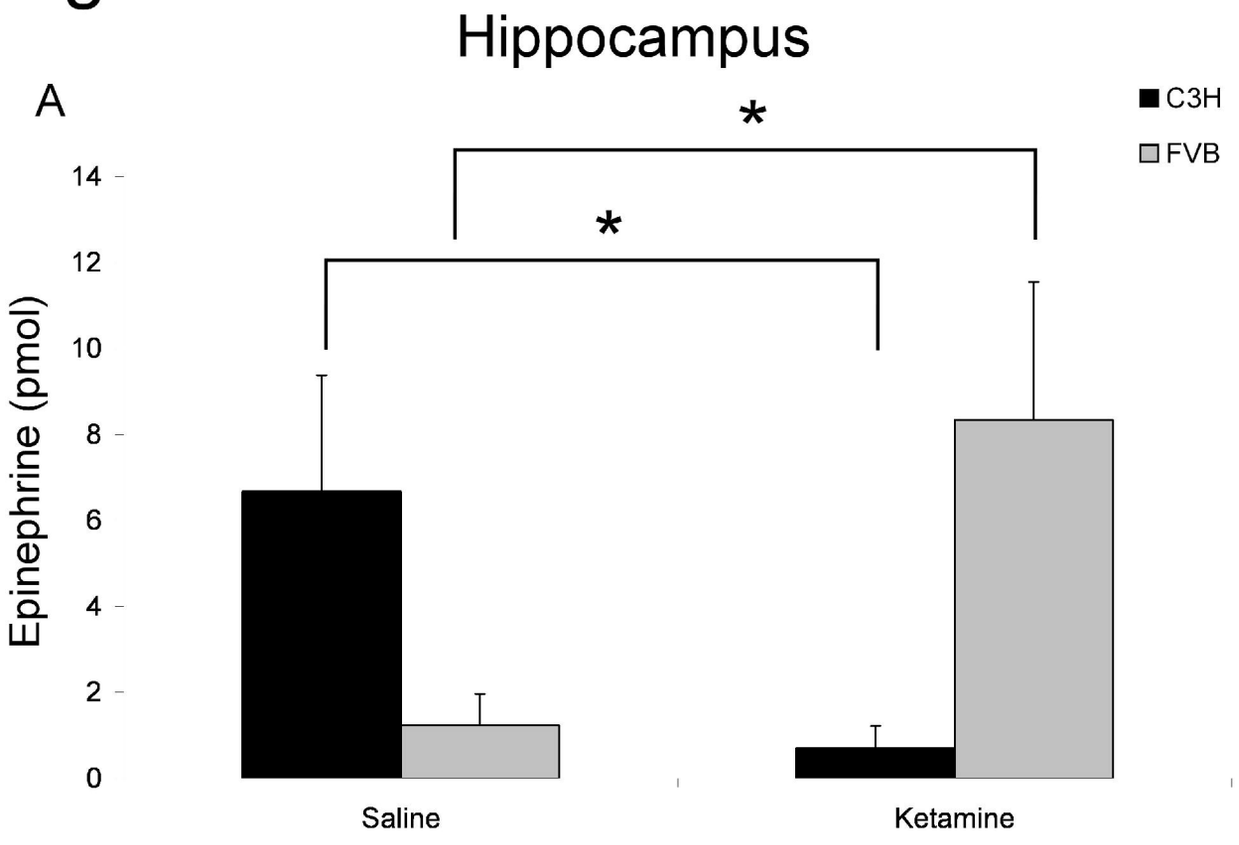

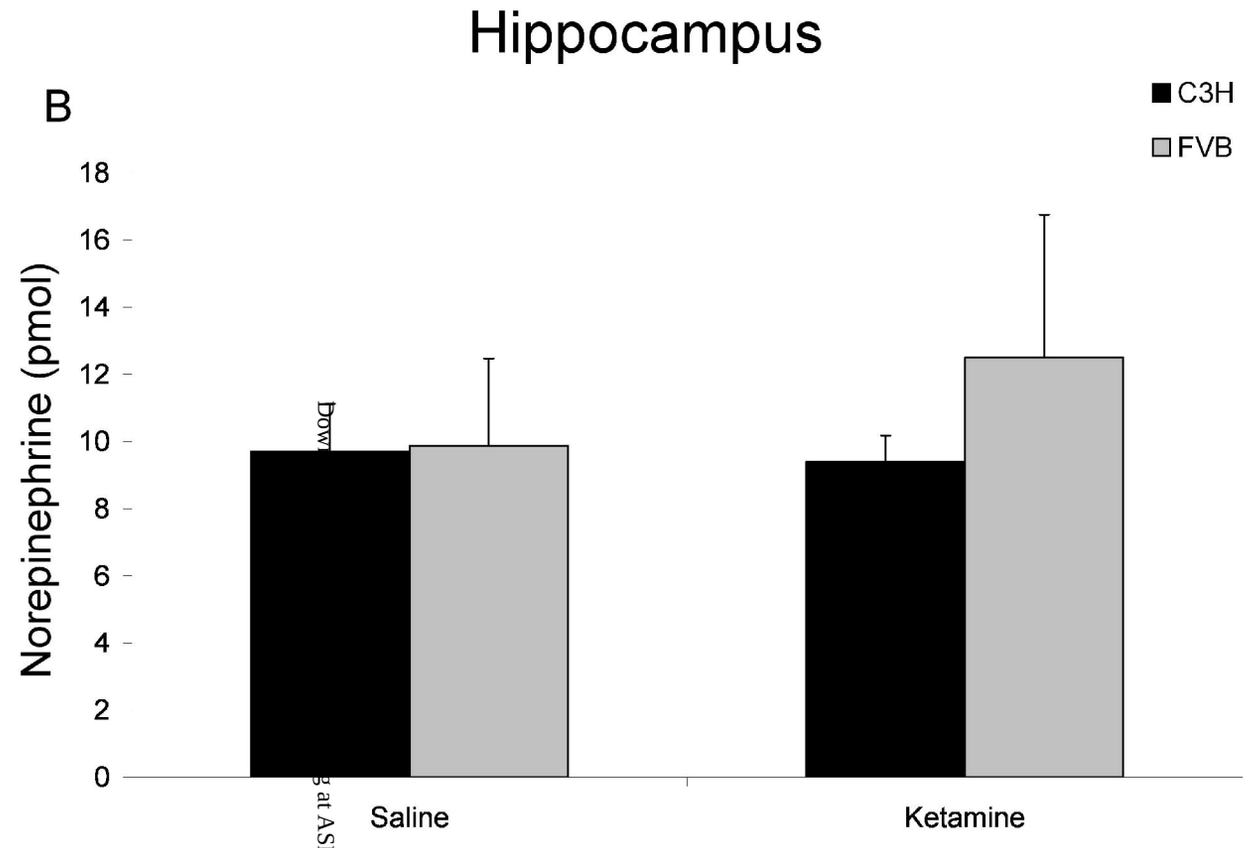

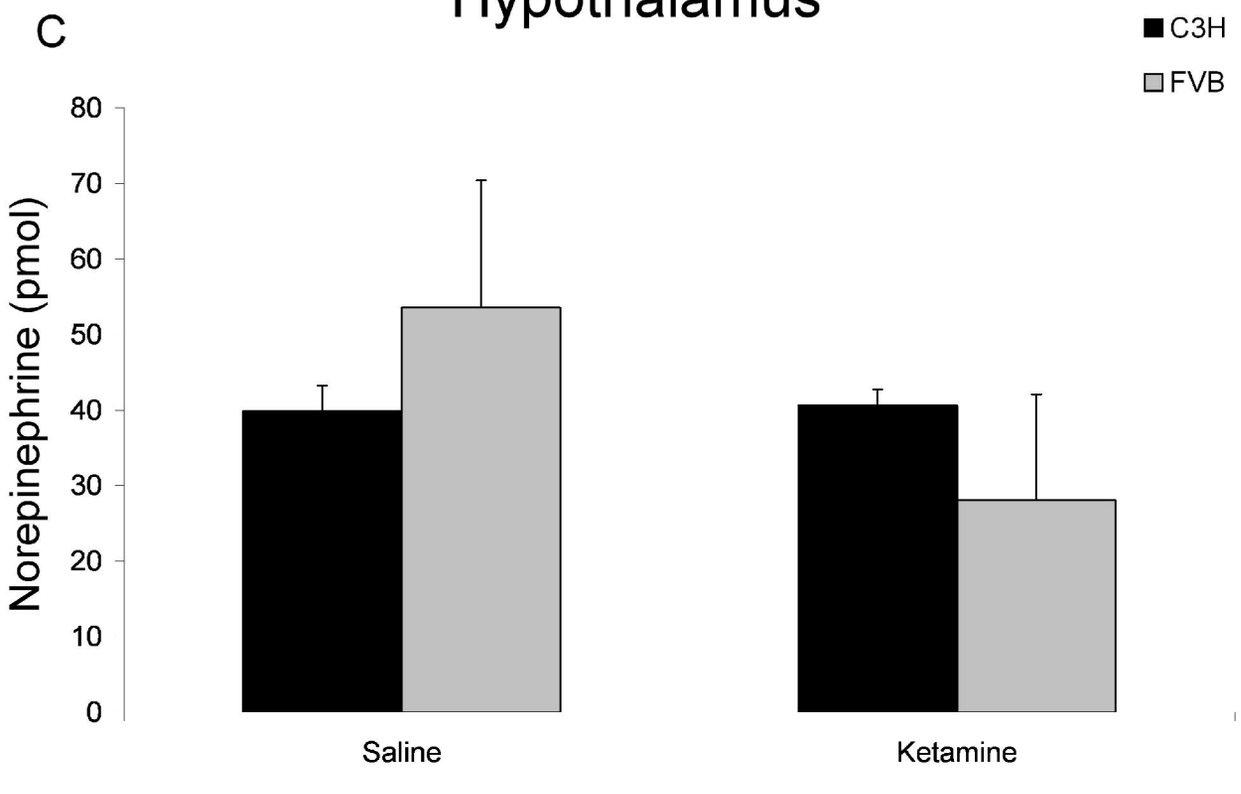

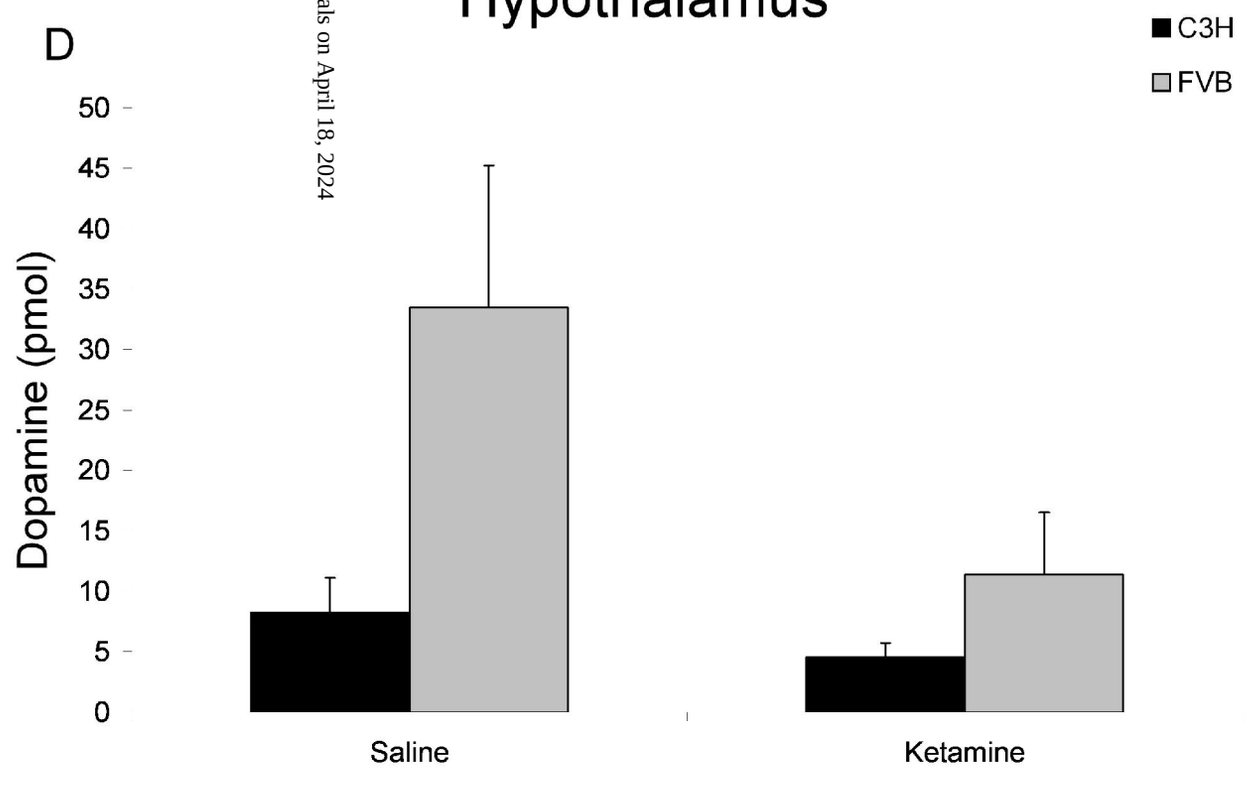

Figure 6

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