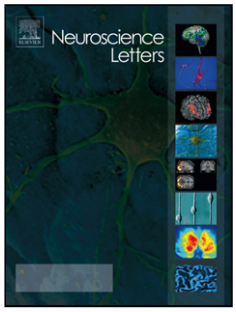

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Ketamine promotes increased freezing behavior in rats with experimental PTSD without changing brain glucose metabolism or BDNF

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Highlights

- Ketamine increases freezing behavior in rats with experimental PTSD
- PTSD and ketamine treatment do not alter BDNF protein levels in rat brain
- PTSD and ketamine treatment do not alter glucose metabolism in rat brain

ABSTRACT

Acute treatment with ketamine, an NMDA receptor antagonist, has been reported to be efficacious in treating depression. The goal of our study was to evaluate ketamine treatment in an animal model of another important psychiatric disease, post-traumatic stress disorder (PTSD). Fifty-eight male rats were initially divided into four groups: Control+Saline (CTRL+SAL), Control+Ketamine (CTRL+KET), PTSD+Saline (PTSD+SAL) and PTSD+Ketamine (PTSD+KET). To mimic PTSD we employed the inescapable footshock protocol. The PTSD animals were classified according to freezing behavior duration into "extreme behavioral response" (EBR) or "minimal behavioral response" (MBR). Afterwards, the glucose metabolism and BDNF were evaluated in the hippocampus, frontal cortex, and amygdala. Our results show that animals classified as EBR exhibited increased freezing behavior and that ketamine treatment further increased freezing duration. Glucose metabolism and BDNF levels showed no significant differences. These results suggest ketamine might aggravate PTSD symptoms and that this effect is unrelated to alterations in glucose metabolism or BDNF protein levels.

Keywords: PTSD; frontal cortex; hippocampus; amygdala; microPET; BDNF

INTRODUCTION

The Diagnostic and Statistical Manual for Mental Disorders (DSM-5) lists PTSD as a condition related to exposure to a traumatic experience. A traumatic experience is characterized by its ability to induce helplessness, horror and/or fear. Generally, PTSD occurs after a life-threatening event, loss of physical integrity or serious injury [1,2]. Some of the better known psychophysiological symptoms of PTSD include exaggerated startle, impaired sleep, intrusive memories or flashbacks and the persistent avoidance of

trauma associated situations/stimuli [2]. Not all individuals confronted with severe traumatic events develop PTSD. Data on PTSD prevalence in the general population vary from 3 to 8% [3,4]. However, Kessler *et al.*, [5] reported that 50% of women and 60% of men will have a traumatic experience at some point in their lives. Therefore, this pathology affects a subpopulation of vulnerable individuals exposed to a traumatic experience that exceeds their capacity to cope [1,2]. Like humans, animals show individual differences in their susceptibility to traumatic stress. Some animals exhibit shorter duration reactions that do not induce prolonged stress responses while others, in spite of being submitted to similar stress situations, exhibit an exacerbated stress response [6].

Ketamine is a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor for glutamate [7]. It has been used clinically since the 1960s as a dissociative anesthetic agent, although its usage has largely been discontinued due to undesired psychic effects (perceptual alterations such as dissociative experiences), occurring in approximately 12% of patients [8]. However, more recently, ketamine has been shown to produce rapid antidepressant effects as well as decreased suicidal ideation following a single sub-anesthetic dose in depressed patients [9,10]. In PTSD patients, the results obtained with ketamine are controversial. Some studies have demonstrated positive effects [11-14], while others have demonstrated negative effects [7,15-17] of ketamine treatment. However, in most cases, ketamine was administered concomitantly with other medications (benzodiazepines, for example). Therefore, it is important to undertake pre-clinical studies to evaluate the independent effects of ketamine.

The most widely accepted hypothesis regarding the mechanisms by which ketamine produces its effects suggests the ketamine-induced NMDA receptor blockade partially impedes activation of eukaryotic elongation factor-2 (eEF2) phosphorylation

and consequently reduces the inhibition of BDNF translation. The restoration of BDNF translation promotes the activation of a signaling cascade, resulting in increased synaptic protein and spine density. Therefore, BDNF is thought to have a central role in the action mechanism of ketamine [18]. Moreover, recent neuroimaging studies suggest the fear neurocircuitry may be altered in PTSD. Two of the main symptoms of PTSD: conditioned fear and fear generalization (the transfer of fear experienced during a traumatic event to safe conditions 'resembling' the traumatic event), are associated with changes in the activity of the hippocampus, amygdala and frontal cortex [19-21].

In this study, we employed the inescapable footshock protocol as an experimental model of PTSD and the population of stress-exposed rodents was classified according to their individual behavioral response (duration of freezing behavior) [6]. We classified the PTSD animals into two distinct groups: "extreme behavioral response" (EBR) and "minimal behavioral response" (MBR). The goals of this study were to evaluate the brain activity through the ^{18}F-2-fluoro-2-deoxy-glucose (^{18}F-FDG) uptake and quantify brain derived neurotrophic factor (BDNF) protein in the hippocampus, amygdala and frontal cortex.

EXPERIMENTAL PROCEDURES

Animals

All procedures were approved by the University's ethical committee (CEUA 13/00350-PUCRS) and were conducted in accordance with the University's guidelines. Fifty-eight, 12-week-old, male Wistar rats were obtained from the *Centro de Modelos Biológicos Experimentais (CeMBE)* of the *Pontifícia Universidade Católica do Rio Grande do Sul.* The rats were kept in standard laboratory conditions with freely available food and water, and a 12:12h dark/light cycle. In the first part of this study, animals were divided into four groups: 1-Control+Saline (CTRL+SAL, n=14); 2-Control+Ketamine

(CTRL+KET, n=14); 3-PTSD+Saline (PTSD+SAL, n=15); 4-PTSD+Ketamine (PTSD+KET, n=15). Then, after the situational reminder test, the animals from groups 3 and 4 were divided into EBR or MBR according to the behavioral criterion. In the PTSD+SAL group, eight animals were classified as EBR and seven animals as MBR, while in the PTSD+KET group, ten animals were classified as EBR and five animals as MBR.

The PTSD experimental model

In this experimental model, the animals were subjected to a single inescapable footshock [1]. The apparatus consists of a 50x25x25 cm box separated into two compartments by a removable door. To induce PTSD, the animals were individually positioned in the first compartment, which has a wooden floor. After two minutes, the door was opened and we waited until the animal crossed into the second compartment, in which the floor is a bronze grid. At that moment, the door was closed and then a 1mA 60Hz footshock was delivered for 20 seconds. The animals in the control groups were subjected to the same procedure, but no shock was applied.

Drug Administration

A single intraperitoneal injection was applied on the night of the 6^{th} day of the experiment (Fig. 1). The ketamine group animals received 10 mg/kg of ketamine (Cristália, Brazil), while the saline group animals received 0.5 ml of saline solution. We chose this ketamine dose, because it has been demonstrated to produce acute and long-lasting antidepressant effects in protocols that mimic depression in animals [22,23,24,45]. The ketamine was administered on 6^{th} day to evaluate the short-term effects of the drug and at the same time to ensure the animals were not subject to a ketamine-induced anesthetic effect.

Situational reminder (SR)

One week after the initial footshock protocol (7th day of the experiment – Fig. 1), the animals were exposed to the SR. The animals were positioned in the first compartment of the apparatus for two minutes, but the door dividing the compartments remained closed. In this behavioral test, two experienced researchers evaluated the length of the freezing bout (the average of both time periods provided the value for each animal), which is a measure of conditioned fear.

Cut-off behavioral criterion

Individual animals from the PTSD+SAL and PTSD+KET groups were classified as having either "extreme" or "minimal" behavioral responses according to a pre-established criterion. Here, the average length of the freezing bout of all the animals in the PTSD+SAL group (mean=14.23 seconds) was used as the cut-off criterion: animals that froze for over 14.23 seconds were classified as EBR, while animals that froze for less than 14.23 seconds were classified as MBR.

^{18}F-FDG MicroPET Scan

Following the behavioral analysis, some animas were randomly selected for ^{18}F-FDG analysis. Only animals from the CTRL+SAL n=5; CTRL+KET n=5; PTSD+SAL(EBR) n=4; and PTSD+KET(EBR) n=5 groups were submitted to the microPET scanning procedure. On day 8 (Fig. 1), the animals received an intravenous injection of 1 mCi of ^{18}F-FDG, and were scanned 40 minutes after conscious tracer uptake. List mode static acquisitions were acquired for 30 minutes using a TriumphTM microPET system (LabPET-4, TriFoil Imaging, Northridge, CA, USA). During the scan, the animals were kept under inhalatory anesthesia (induction at 3–4% isoflurane and medical oxygen, and 2–3% for maintenance dose), with body temperature maintained at 36ºC. The field of view (FOV; 3.75cm) was centered on the rat's head. Data were

reconstructed using the 3D-MLEM algorithm (3D maximum likelihood estimation method) with 20 iterations. The images were not corrected for attenuation. The Fusion Toolbox (PMOD v3.5, PMOD Technologies, Zurich, Switzerland) was used to spatially normalize the microPET images into an ^{18}F-FDG template. An MRI rat brain VOI template, previously co-registered to the microPET image database, was used to overlay the normalized images [24]. The ^{18}F-FDG uptake in the frontal cortex, hippocampus and amygdala were expressed as standard uptake values (SUVs).

Sample extraction and BDNF determination

To analyze BDNF in the cerebral cortex, the number of animals used from each group was: CTRL+SAL n=13; CTRL+KET n=13; PTSD+SAL(EBR) n=8; PTSD+SAL(MBR) n=7; PTSD+KET(EBR) n=10; and PTSD+KET(MBR) n=4; and in hippocampus the number of animals in each group was: CTRL+SAL n=14; CTRL+KET n=14; PTSD+SAL(EBR) n=8; PTSD+SAL(MBR) n=7; PTSD+KET(EBR) n=9; and PTSD+KET(MBR) n=4. On day 9 of the experiment (Fig. 1), the animals were decapitated without anesthesia. The rat brains were immediately removed and washed in saline solution. The frontal cortex and hippocampus were dissected out, placed in liquid nitrogen and stored at -80ºC until used. For technical reasons, the amygdala was not dissected. The BDNF concentration was determined by ELISA according to the manufacturer's instructions (Millipore, Sandwich ELISA Kit, ChemiKine^{TM}).

Data analysis

The data were analyzed using: 1-Pearson's test to evaluate the correlation between the freezing bouts counted by both observers during the SR; 2-Two-way ANOVA, followed by Tukey *post hoc* tests - to evaluate interactions between "disease status" and "treatment" and differences between groups. The analyses were performed using SPSS 17.0 software. Results are presented as mean±SE. ($p \leq 0.05$).

RESULTS

Freezing behavior

Freezing behavior during the SR is shown in Fig. 2. The analysis of Pearson's correlation coefficient revealed a strong correlation between the observers ($r = 0.981$; $p<0.001$). The two way ANOVA revealed a significant "disease status" effect ($F_{(2,58)}=52.57$; $p<0.001$) and a "disease status" X "drug treatment" interaction ($F_{(2,58)}=6.45$; $p=0.003$) and there was no ketamine effect *per se* ($F_{(2,58)}=1.94$; $p=0.17$). Tukey *post hoc* tests revealed the EBR animals (from both the PTSD+SAL and PTSD+KET groups) presented longer freezing bouts when compared with all the other groups ($p<0.01$). Additionally, animals from the PTSD+KET(EBR) group exhibited longer freezing bouts when compared with animals from the PTSD+SAL(EBR) group ($p<0.01$). Thus, we observe that our PTSD protocol induced freezing behavior and ketamine interacted positively with this effect, promoting a prolonged freezing bout.

^{18}F-FDG-MicroPET

The glucose metabolism induced by PTSD, as well as the effect of ketamine treatment are shown in Fig. 3. The ^{18}F-FDG SUVs showed no significant statistical differences for "disease status" in the frontal cortex ($p=0.951$; $F_{(1,15)}=0.004$) (Fig. 3a and 3b), hippocampus ($p=0.858$; $F_{(1,15)}=0.033$) (Fig. 3d and 3e) or amygdala ($p=0.752$; $F_{(1,15)}=0.103$) (Fig 3g and 3h). Additionally, no effects were found for "drug treatment" in the frontal cortex ($p=0.637$; $F_{(1,15)}=0.231$), hippocampus ($p=0.463$; $F_{(1,15)}=0.565$) or amygdala ($p=0.426$; $F_{(1,15)}=0.667$). No interaction between "disease status" and "drug treatment" was found.

BDNF protein levels

The effects of PTSD and ketamine treatment on BDNF protein levels are shown in Fig. 3. No significant differences were observed for "disease status" in the frontal cortex

(p=0.051; F(2,49)=20.963) (Fig. 3c) or hippocampus (p=0.365; F(2,50)=1.028) (Fig. 3f). Moreover, no additional differences were found for "drug treatment" in the frontal cortex (p=0.538; F(1,49)=0.384) or hippocampus (p=0.822; F(1,50)=1.339). No interaction between "disease status" and "drug treatment" was found.

DISCUSSION

In this study, we observed that an inescapable footshock protocol as an experimental model of PTSD and treatment with ketamine did not alter the resting glucose metabolism or BDNF protein in the frontal cortex, hippocampus or amygdala of Wistar rats. Moreover, we noted that ketamine treatment increased freezing behavior in the EBR animals.

Our results demonstrate that, as observed in humans, animals show consistent individual differences in their behavioral and physiological response patterns to environmental demands. Regarding freezing behavior, we observed that the PTSD animals classified as MBR presented similar responses to the CTRL+SAL group (Fig. 2). As reported in other studies, not all stressed animals responded similarly. Some remained unaffected, showing little fear sensitization [25,26]. Even in highly inbred laboratory strains, genetically identical subjects may be considerably more or less susceptible to similar experimental manipulations [27]. The reasons for these individual differences remain largely unknown, although individual neurobiology and past experience are considered a major risk factor for the development of the disease.

Unlike the MBR animals, the EBR animals (both PTSD+SAL and PTSD+KET) showed longer freezing behavior during the SR when compared to all other groups (Fig. 2). Freezing behavior is used as a measure of conditioned fear and indicates a sense of intense horror or immediate threat. This behavior suggests the animals developed long-lasting anxiety, which is one of the main features of PTSD, because the footshock was

remembered 7 days after the exposure. Moreover, the animals from the PTSD+KET group exhibited longer freezing bouts when compared to the PTSD+SAL group. These data demonstrate that, at least in animals exposed to stress, ketamine worsens anxiety-related behavior. The same result has been described in an experimental PTSD study involving predator-scent stress, which showed that exposed rats treated with 3 different doses of ketamine (0.5, 5 and 15mg/kg administered one hour following stress exposure) exhibited a significant increase in freezing behavior [7]. A human study demonstrated that 15 trauma-exposed burn subjects who received ketamine/midazolam as an analgesic/sedative treatment presented significantly more severe PTSD symptoms than subjects who were not given the treatment [15]. Similar results have been described in previous studies involving victims of moderate accidents. Patients who received ketamine during their initial emergency treatment showed an increase in re-experiencing symptoms, elevated dissociative symptoms and heightened avoidance when compared to patients who received opioid medication during their initial treatment [16,17]. Ketamine is an anesthetic administered especially in military hospitals to burns patients. This drug is associated with psychosis and dissociation, leading to the concern that it may increase the rates of PTSD development [28]. Moreover, some authors suggest that antagonizing the NMDA receptor increases the vulnerability of stressed patients to develop PTSD, because clinical studies propose that NMDA antagonists may transiently stimulate cortico-limbic glutamate release and produce symptoms resembling dissociative states [15,29]. However, there is no consensus regarding these data, because studies conducted in humans and animals have demonstrated beneficial effects of ketamine in the treatment of PTSD symptoms [11-14].

Because the frontal cortex, hippocampus and amygdala are the three major regions involved in the stress response, we decided to analyze the glucose metabolism and BDNF

levels in these regions. In our study, we found no change in BDNF expression in any of the analyzed regions nine days after the stress exposure. Our results are in accordance with those reported by previous experimental studies that analyzed the effects of PTSD on long- and short-term BDNF mRNA expression, which showed alterations in BDNF levels are transient and restricted to an hour or a few days [30-33]. Similarly, a study involving PTSD patients found higher BDNF serum levels in the PTSD group. However, that study also showed that patients who were subjected to trauma exposure in the previous year maintained that difference, while those patients with more remote trauma (more than 1 year) did not [34]. The only study exploring BDNF levels in the cerebrospinal fluid of PTSD patients found no difference when compared with controls [35]. In our study, we evaluated the BDNF levels 9 days after the initial stress. Taken together, the above findings suggest that stress events may dysregulate BDNF signaling, perhaps transiently, thus interfering with the normal functioning of the brain and contributing to the potential development of PTSD.

The brain glucose metabolism was analyzed using the ^{18}F-FDG-microPET technique, which measures the glucose uptake in tissues. In our study, no apparent alterations were observed in the frontal cortex, hippocampus or amygdala. Extant data demonstrating ^{18}F-FDG-microPET or even cerebral blood flow (CBF) abnormalities in PTSD patients/animal models are limited and conflicting. For example: increased [36-39], decreased [36,40,41] and unchanged [42,43] glucose metabolism and CBF have been reported in different nuclei within the amygdala. Similar conflicting results have also been found in different areas of the PFC, with increased [39,44] and decreased [39,41-44] glucose metabolism and CBF being reported. The only consensus seems to be with respect to the hippocampus, in which only decreases in glucose metabolism have been reported [36,41,42]. These contradictory results can be attributed to many factors, such

as differences in time since the onset of PTSD, heterogeneity of trauma exposure and differences in the paradigms in which the analyses were performed (resting, sleep, trauma-related, trauma-unrelated and neutral scripts). Therefore, it seems that due to PTSD's complex clinical presentation and quite variable symptomatology, it is difficult to establish consistent data about ^{18}F-FDG in this neuropsychiatric condition. Thus, more standardized protocols and nosological classifications of the disease may help clarify these discrepancies.

Other important points should be considered in relation to the effects of ketamine. The classic pathway related to the antidepressant properties of ketamine involves NMDA receptor blockage. However, recent studies have shown that ant depressive effects are in fact generated by ketamine metabolites, (2R,6R)-hydroxynorketamine (HNK), which are responsible for a sustained activation of α-amino-3-hydroxy-5-methyl1-4isoxazole propionic acid (AMPA) receptors [45,46]. Currently, it is unknown whether the increase duration of freezing bouts found in animals from the PTSD+KET group was associated to NMDA receptor blockage by ketamine or AMPA receptor activation by HNK, or perhaps related to other effects of ketamine and its metabolites in other receptors, such as the: opioid, muscarinic, nicotinic, dopaminergic and serotoninergic receptors [46]. Further studies, testing the effects of ketamine and its metabolites in PTSD models and analyzing different neurotransmission systems, could clarify this point.

Another suggestion for further research is to test PTSD and treatment with ketamine and its metabolites in female rats, since such females have been shown to present an enhanced ant depressive response when compared to males submitted to the same treatment [45]. The same study shows that, after ketamine treatment, the level of HNK is three times higher in the female brain compared to male [45].

Another interesting topic for future studies about the effects of ketamine in PTSD would be related to the density of stubby and mushroom spines, since a study with postmortem PTSD shows that PTSD promotes an increase in stubby spine density and a trend towards a reduction in mushroom spine density in medial orbital cortex [47]. The same change in spine density was found in the hippocampus of rats using another NMDA receptor antagonist, MK-801 [48]. Indicating that PTSD and NMDA blockage could corroborate to similar changes in dendritic spines.

In summary, the main findings of our study are that acute ketamine treatment increases freezing behavior in PTSD animals. However, this alteration seems to be unrelated to changes in glucose metabolism or BDNF levels in the hippocampus, frontal cortex or amygdala. Moreover, further studies are necessary to better understand the effects of ketamine in PTSD and other psychiatric diseases.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

[1]Diehl LA, Silveira PP, Leite MC, Crema LM, Portella AK, Billodre MN, Nunes E, Henriques TP, Fidelix-da-Silva LB, Heis MD, Gonc¸alves CA, Quillfeldt JA, Dalmaz C (2007) Long lasting sex-specific effects upon behavior and S100b levels after maternal separation and exposure to a model of post-traumatic stress disorder in rats. Brain Res 1144:107–116

[2]Liberzon I, Khan S, Young EA (2005) Animal models of posttraumatic stress disorder. Handb Stress Brain 15:231–250

[3]Bisson JI, Cosgrove S, Lewis C, Robert NP (2015) Post-traumatic stress disorder. BMJ 26;351:h6161

[4]Yehuda R (2002) Post-traumatic stress disorder. N Engl J Med 346(2):108-14

[5]Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB (1995) Posttraumatic stress disorder in the National Comorbidity Survey. Arch Gen Psychiatry 52(12):1048-1060

[6]Cohen H, Kozlovsky N, Matar MA, Zohar J, Kaplan Z (2014) Distinctive hippocampal and amygdalar cytoarchitectural changes underlie specific patterns of behavioral

disruption following stress exposure in an animal model of PTSD. Eur Neuropsychopharmacol 24:1925–1944
[7]Juven-Wetzler A, Cohen H, Kaplan Z, Kohen A, Porat O, Zohar J (2014) Immediate ketamine treatment does not prevent posttraumatic stress responses in an animal model for PTSD. Eur Neuropsychopharmacol 24(3):469-79
[8]Annetta MG, Iemma D, Garisto C, Tafani C, Proietti R (2005) Ketamine: new indications for an old drug. Curr Drug Targets 6(7):789-94
[9]aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS, Mathew SJ (2010) Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. Biol Psychiatry 67(2):139-145
[10]Zarate CA Jr, Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A, Selter J, Marquardt CA, Liberty V, Luckenbaugh DA (2012) Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. Biol Psychiatry 71(11):939-946
[11]McGhee LL, Maani CV, Garza TH, Gaylord KM, Black IH (2008) The correlation between ketamine and posttraumatic stress disorder in burned service members. J Trauma 64:S195-199
[12]Feder A, Parides MK, Murrough JW, Perez AM, Morgan JE, Saxena S, Kirkwood K, Aan Het Rot M, Lapidus KA, Wan LB, Iosifescu D, Charney DS (2014) Efficacy of intravenous ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. JAMA Psychiatry 71(6):681-8
[13]D'Andrea D, Sewell RA (2013) Transient resolution of treatment-resistant posttraumatic stress disorder following ketamine infusion. Biol Psychiatry 74(9):e13-4
[14]Zhang LM, Zhou WW, Ji YJ, Li Y, Zhao N, Chen HX, Xue R, Mei XG, Zhang YZ, Wang HL, Li YF (2015) Anxiolytic effects of ketamine in animal models of posttraumatic stress disorder. Psychopharmacology 232(4):663-72
[15]Winter H, Irle E (2004) Hippocampal volume in adult burn patients with and without posttraumatic stress disorder. Am J Psychiatry 161(12):2194-200
[16]Schönenberg M, Reichwald U, Domes G, Badke A, Hautzinger M (2005) Effects of peritraumatic ketamine medication on early and sustained posttraumatic stress symptoms in moderately injured accident victims. Psychopharmacology 182(3):420-5
[17]Schönenberg M, Reichwald U, Domes G, Badke A, Hautzinger M (2008) Ketamine aggravates symptoms of acute stress disorder in a naturalistic sample of accident victims. J Psychopharmacol 22(5):493-7
[18]Naughton M, Clarke G, O'Leary OF, Cryan JF, Dinan TG (2014) A review of ketamine in affective disorders: current evidence of clinical efficacy, limitations of use and pre-clinical evidence on proposed mechanisms of action. J Affect Disord 156:24-35
[19]Liberzon I, Taylor SF, Amdur R, Jung TD, Chamberlain KR, Minoshima S, Koeppe RA, Fig LM (1999) Brain activation in PTSD in response to trauma-related stimuli. Biol Psychiatry 1;45(7):817-26
[20]Shin LM, Rauch SL, Pitman RK (2006) Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. Ann N Y Acad Sci 1071:67-79
[21]Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, Peters PM, Metzger LJ, Dougherty DD, Cannistraro PA, Alpert NM, Fischman AJ, Pitman RK (2004) Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. Arch Gen Psychiatry 61(2):168-76
[22]Kristal JH, Sanacora G, Duman RS (2013) Rapid-actin glutamatergic antidepressant: the path to ketamine and beyond. Biol Psychiatry 73:1133-1141

[23]Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329:959–964
[24]Baptista PP, Saur L, Bagatini PB, Greggio S, Venturin GT, Vaz SP, Ferreira Kdos R, Junqueira JS, Lara DR, DaCosta JC, Jeckel CM, Mestriner RG, Xavier LL (2015) Antidepressant Effects of Ketamine Are Not Related to ^{18}F-FDG Metabolism or Tyrosine Hydroxylase Immunoreactivity in the Ventral Tegmental Area of Wistar Rats. Neurochem Res 40(6):1153-64
[25]Cohen H, Zohar J, Matar M (2003) The relevance of differential response to trauma in an animal model of posttraumatic stress disorder. Biological Psychiatry 53(6):463–73
[26]Cohen H, Zohar J, Matar MA, Zeev K, Loewenthal U, Richter-Levin G (2004) Setting apart the affected: the use of behavioral criteria in animal models of post traumatic stress disorder. Neuropsychopharmacology 29(11):1962–70
[27]Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131(2):391-404
[28]Cukor J, Spitalnick J, Difede J, Rizzo A, Rothbaum BO (2009) Emerging treatments for PTSD. Clin Psychol Rev 29(8):715-726
[29]Chambers RA, Bremner JD, Moghaddam B, Southwick SM, Charney DS, Krystal JH (1999) Glutamate and post-traumatic stress disorder: toward a psychobiology of dissociation. Semin Clin Neuropsychiatry (4):274-81
[30]Rasmusson AM, Shi L, Duman R (2002) Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. Neuropsychopharmacology 27(2):133-42
[31]Kozlovsky N, Matar MA, Kaplan Z, Kotler M, Zohar J, Cohen H (2007) Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. Int J Neuropsychopharmacol 10(6):741-58
[32]Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA (2004) Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol Psychiatry 55:708–714
[33]Bland ST, Schmid MJ, Der-Avakian A, Watkins LR, Spencer RL, Maier SF (2005) Expression of c-fos and BDNF mRNA in subregions of the prefrontal cortex of male and female rats after acute uncontrollable stress. Brain Res 1051(1-2):90-9
[34]Hauck S, Kapczinski F, Roesler R, de Moura Silveira E Jr, Magalhães PV, Kruel LR, Schestatsky SS, Ceitlin LH (2010) Serum brain-derived neurotrophic factor in patients with trauma psychopathology. Prog Neuropsychopharmacol Biol Psychiatry 34(3):459-62
[35]Bonne O, Gill JM, Luckenbaugh DA, Collins C, Owens MJ, Alesci S, Neumeister A, Yuan P, Kinkead B, Manji HK, Charney DS, Vythilingam M (2011) Corticotropin-releasing factor, interleukin-6, brain-derived neurotrophic factor, insulin-like growth factor-1, and substance P in the cerebrospinal fluid of civilians with posttraumatic stress disorder before and after treatment with paroxetine. J Clin Psychiatry 72(8):1124-8
[36]Yehuda R, Harvey PD, Golier JA, Newmark RE, Bowie CR, Wohltmann JJ, Grossman RA, Schmeidler J, Hazlett EA, Buchsbaum MS (2009) Changes in relative glucose metabolic rate following cortisol administration in aging veterans with posttraumatic stress disorder: an FDG-PET neuroimaging study. J Neuropsychiatry Clin Neurosci 21(2):132-43

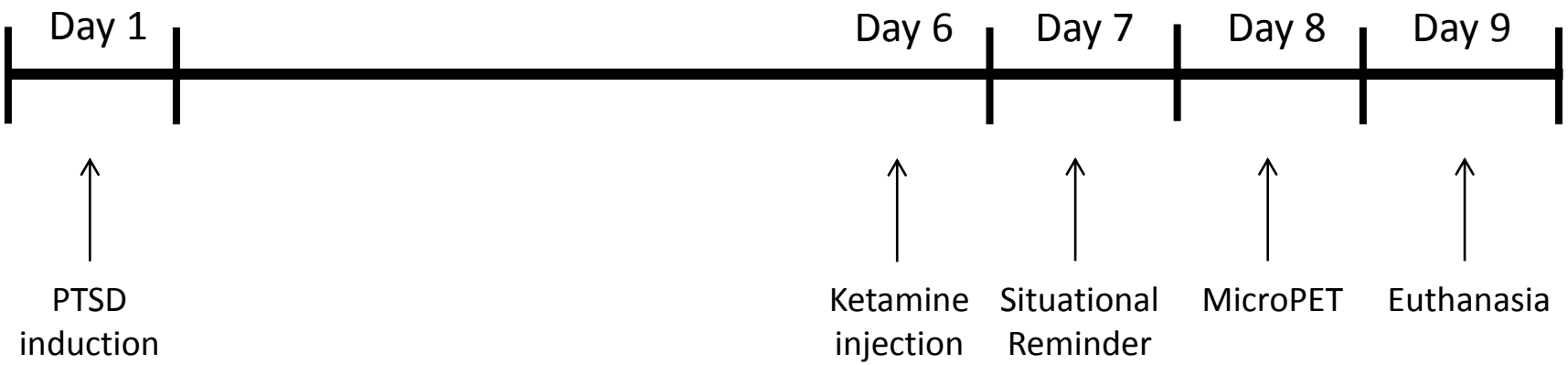
[37]Ramage AE, Litz BT, Resick PA, Woolsey MD, Dondanville KA, Young-McCaughan S, Borah AM, Borah EV, Peterson AL, Fox PT (2016) Regional cerebral glucose metabolism differentiates danger- and non-danger-based traumas in post-traumatic stress disorder. Soc Cogn Affect Neurosci 11(2):234-42
[38]Zhu Y, Du R, Zhu Y, Shen Y, Zhang K, Chen Y, Song F, Wu S, Zhang H, Tian M (2016) PET Mapping of Neurofunctional Changes in a Post-traumatic Stress Disorder Model. J Nucl Med Mar 16. pii: jnumed.116.173443
[39]Vermetten E, Schmahl C, Southwick SM, Bremner JD (2007) Positron tomographic emission study of olfactory induced emotional recall in veterans with and without combat-related posttraumatic stress disorder. Psychopharmacol Bull 40(1):8-30
[40]Buchsbaum MS, Simmons AN, DeCastro A, Farid N, Matthews SC (2015) Clusters of Low (18)F-Fluorodeoxyglucose Uptake Voxels in Combat Veterans with Traumatic Brain Injury and Post-Traumatic Stress Disorder. J Neurotrauma 15;32(22):1736-50
[41]Stocker RP, Cieply MA, Paul B, Khan H, Henry L, Kontos AP, Germain A (2014) Combat-related blast exposure and traumatic brain injury influence brain glucose metabolism during REM sleep in military veterans. Neuroimage 99:207-14
[42]Molina ME, Isoardi R, Prado MN, Bentolila S (2010) Basal cerebral glucose distribution in long-term post-traumatic stress disorder. World J Biol Psychiatry (2 Pt 2):493-501
[43]Gold AL, Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, Metzger LJ, Dougherty DD, Alpert NM, Fischman AJ, Pitman RK (2011) Decreased regional cerebral blood flow in medial prefrontal cortex during trauma-unrelated stressful imagery in Vietnam veterans with post-traumatic stress disorder. Psychol Med 41(12):2563-72
[44]Rilling JK, Winslow JT, O'Brien D, Gutman DA, Hoffman JM, Kilts CD (2001) Neural correlates of maternal separation in rhesus monkeys. Biol Psychiatry 49(2):146-57
[45]Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, Alkondon M, Yuan P, Pribut HJ, Singh NS, Dossou KSS, Fang Y, Huan XP, Mayo C.L, Wainer IW, Albuquerque EX,Thompson SM, Thomas CJ, Zarate Jr CA, Gould TD (2016) NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 533(7604):481-486
[46]Tyler MW, Yourish HB, Ionescu DF, Haggarty SJ (2017) Classics in Chemical Neuroscience: Ketamine. ACS Chemical Neuroscience 8(6):1122-1134
[47] Young KA, Thompson PM, Cruz DA, Williamson DE, Selemon LD (2015)
BA11 FKBP5 expression levels correlate with dendritic spine density in postmortem PTSD and controls. Neurobiology of Stress 2:67-72
[48] Han D, Xu L, Xiao H, Prado Schmidt GC, Shi S (2013) Dizocilpine reduces head diameter of dendritic spines in the hippocampus of adolescent rats. Psychiatry Research 210(1):351-356

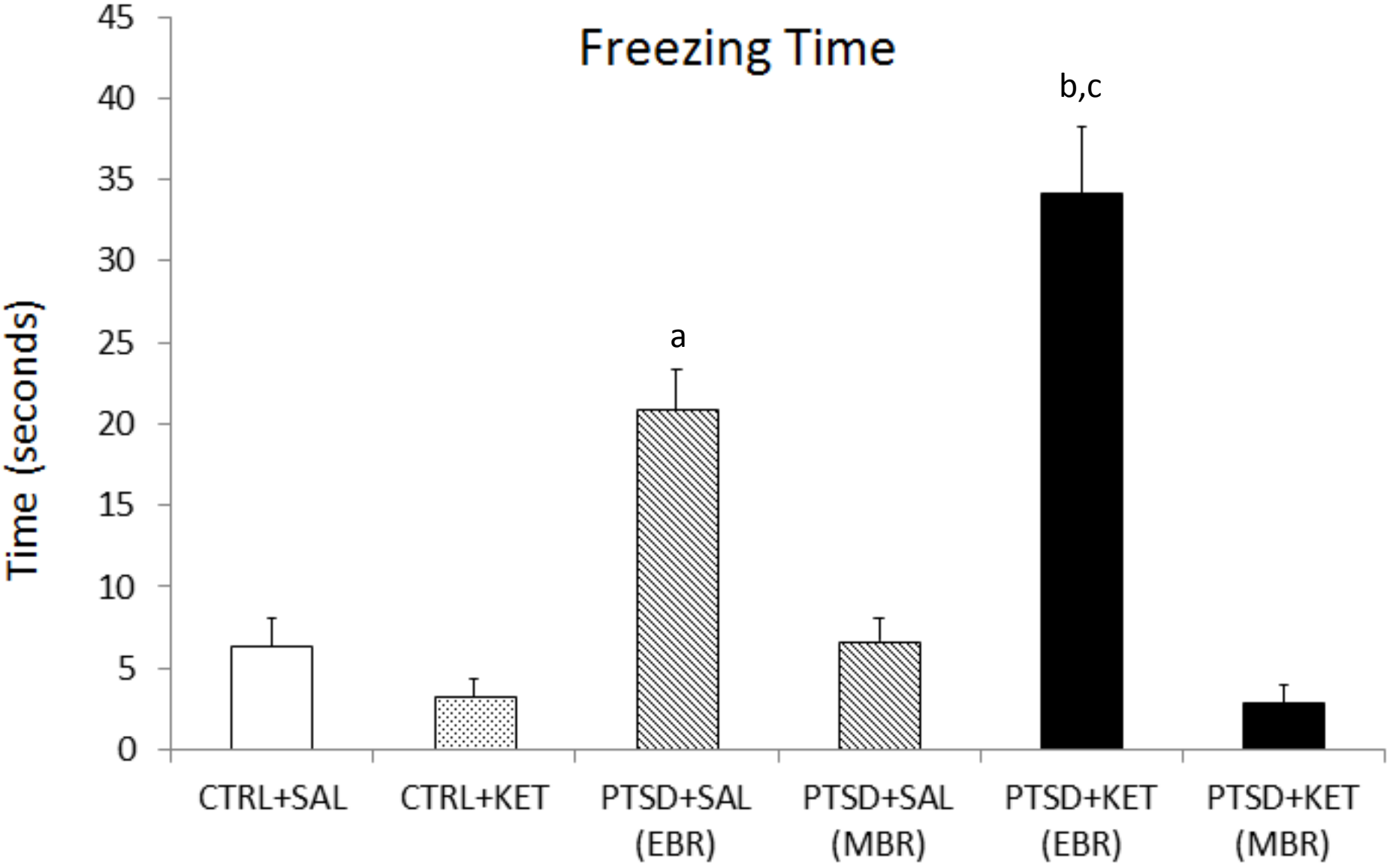
LEGENDS

FIGURE 1 –Timeline depicting the experimental procedure.

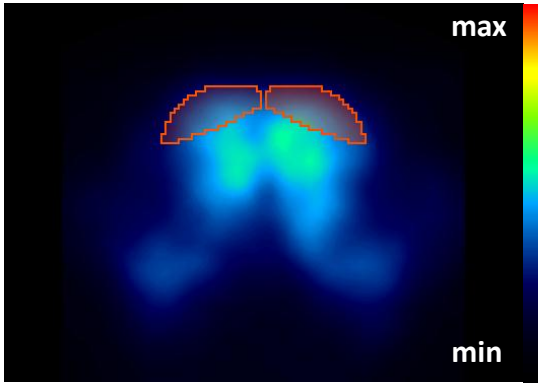
FIGURE 2 –Duration of freezing bouts during exposure to the situational reminder 7 days after PTSD induction. Longer freezing bouts were observed in the PTSD+SAL(EBR) and PTSD+KET(EBR) groups when compared to all other groups. Moreover, the PTSD+KET(EBR) group exhibited longer freezing bouts when compared to the PTSD+SAL(EBR) group. (a) $p \leq 0.01$, when compared to the CTRL+SAL, CTRL+KET, PTSD+SAL(MBR) and the PTSD+KET(MBR) groups; (b) $p \leq 0.01$, when compared to the CTRL+SAL, CTRL+KET, PTSD+SAL(MBR) and PTSD+KET(MBR) groups; (c) $p \leq 0.01$ when compared to the PTSD+SAL(EBR) group.

FIGURE 3 –^{18}F-FDG-microPET and BDNF quantification. Effects of PTSD and ketamine treatment on glucose metabolism and BDNF protein levels in the frontal cortex, hippocampus and amygdala. (a, d, g) Normalized brain image in a coronal view showing the frontal cortex (orange), hippocampus (purple) and amygdala (red), respectively. Areas were defined based on Paxinos coordinates using a rat-ROI-template in the PMOD software (Color figure online). (b, e, h) ^{18}F-FDG uptake in the frontal cortex, hippocampus and amygdala, respectively. (c, f) BDNF protein quantification in the frontal cortex and hippocampus, respectively.

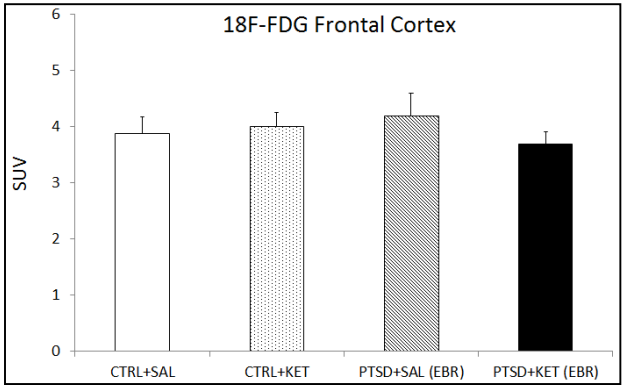

Day 1
Day 6
Day 7
Day 8
Day 9
PTSD induction
Ketamine injection
Situational Reminder
MicroPET
Euthanasia


Freezing Time
Time (seconds)
45
40
35
30
25
20
15
10
5
0
a
b,c
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+SAL (MBR)
PTSD+KET (EBR)
PTSD+KET (MBR)

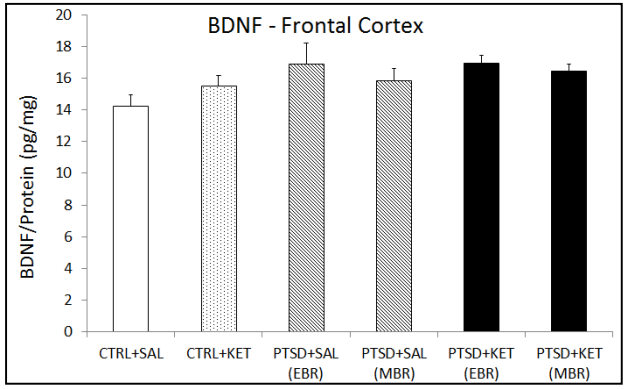
a


max
min

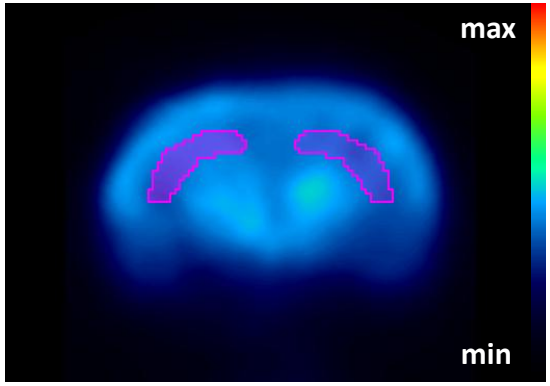
b


18F-FDG Frontal Cortex
SUV
0
1
2
3
4
5
6
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+KET (EBR)

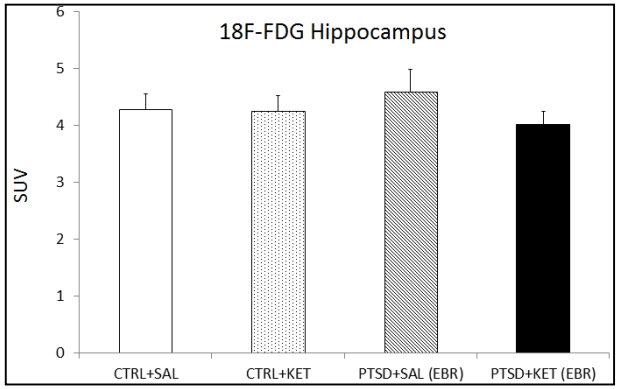
c


BDNF - Frontal Cortex
BDNF/Protein (pg/mg)
0
2
4
6
8
10
12
14
16
18
20
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+SAL (MBR)
PTSD+KET (EBR)
PTSD+KET (MBR)

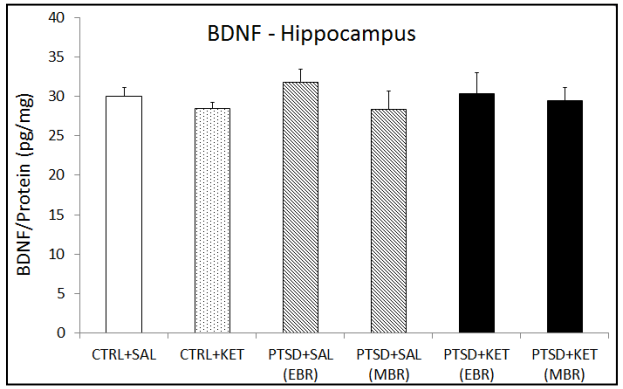
d


max
min

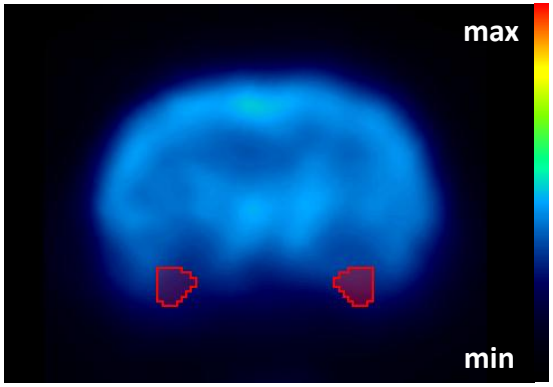
e


18F-FDG Hippocampus
SUV
0
1
2
3
4
5
6
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+KET (EBR)

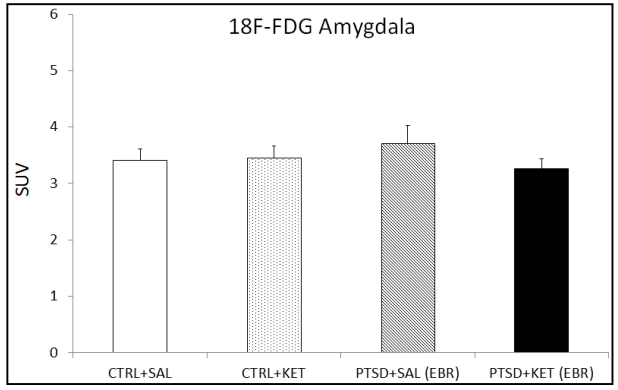
f


BDNF - Hippocampus
BDNF/Protein (pg/mg)
0
5
10
15
20
25
30
35
40
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+SAL (MBR)
PTSD+KET (EBR)
PTSD+KET (MBR)

g


max
min

h


18F-FDG Amygdala
SUV
0
1
2
3
4
5
6
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+KET (EBR)