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Region-specific alteration in brain glutamate: Possible relationship to risk-taking behavior

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ABSTRACT

Risk-taking behaviors involve increased motor activity and reduced anxiety in humans. Total sleep deprivation (SD) in animals produces a similar change in motor and fear behaviors. Investigators studied region-specific brain levels of glutamate in rats after TSD, an animal model of risk-taking behavior. We investigated the effects of sleep deprivation on these behaviors and associated levels of brain glutamate. Compared to the controls, the sleep-deprived rats spent a significantly greater percentage of time in the open arms of the elevated plus maze (EPM), demonstrating reduced fear-like and increased risk-taking behaviors. Additionally, sleep deprivation was associated with a significant increase in glutamate levels in the hippocampus and thalamus. An inverse relationship between glutamate in the medial prefrontal cortex and risk taking in the EPM and a positive association between the ratio of glutamate in the hippocampus to medial prefrontal cortex and risk taking was revealed. The role of sleep deprivation-induced changes in brain glutamate and its relationship to anxiety, fear, and posttraumatic stress disorder (PTSD) is discussed.
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1. Introduction

The brain glutamate system plays an important role in the neurobiology of animal fear and human anxiety. For example, in laboratory animals, the blockade of glutamate neurotransmission results in reduced fear behaviors in the elevated plus maze (EPM) and an attenuated stress-induced increase in acoustic startle [1–5]. Consistent with these data, drugs that attenuate glutamate activity in humans are efficacious in the treatment of anxiety [6–12].

Separate lines of investigation indicate that sleep deprivation, regardless of the sleep restriction methods utilized (i.e., sleep fragmentation, selective REM deprivation, or total sleep restriction), is associated with reduced fear behaviors in animal models of anxiety including the EPM [13,14], open field [15–17], and preference for novelty tests [18]. Given that glutamate blockade and sleep deprivation both reduce fear behaviors, one would expect sleep deprivation to decrease brain glutamate in selective brain regions. While sleep deprivation has been shown to increase glutamate levels in *whole brain* (i.e., cerebral cortex) [19], a seemingly apparent contradiction to the aforementioned effects of glutamatergic blockade on fear/anxiety, no research team has investigated *region-specific* levels of glutamate on fear and risk-taking behaviors.

Risk-taking behavior can be conceptualized as reduced fear and increased (i.e. impulsive) motor activity. Interestingly, sleep deprivation,

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in addition to its effects on fear behavior, has been shown to induce behavioral activation, increasing energy and motor activity, in both depressed humans and animal models [13,14,20–23]. Moreover, the behavioral phenotype displayed in animal models after sleep deprivation is similar to sleep deprivation-induced alterations in activity level, risk taking, decision making, and constructive thinking skills demonstrated by humans [24–28].

The aim of the present study was to assess the behavioral and biochemical correlates of total sleep deprivation (TSD) in rats, with a focus on the relationship between a TSD-induced change in fear/risk-taking behavior, as measured by the EPM, and regional neurochemical concentrations of glutamate (Glu), as measured by high resolution, magic angle spinning proton magnetic resonance spectroscopy (HR-MAS, ^{1}H MRS). We hypothesized that TSD-induced behavioral phenotypes (decreased fearfulness/increased risk taking) would be associated with regional changes in glutamate levels in the cortico-limbic (i.e., fear) circuit.

2. Materials and methods

2.1. Subjects

Thirty-two adult male Sprague–Dawley rats (70 days old) were housed individually in standard, solid bottom cages with ground corncob bedding and ad lib access to food and water. Animals were acclimated to a 23 °C holding room, on a 12 h light/12 h dark cycle, for at least 3 days prior to testing. All rats were handled in accordance with the Penn State College of Medicine Institutional Animal Care and Use Committee (PSCOM-IACUC) policy, which is in agreement with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

The elevated plus maze (San Diego Instruments, San Diego, CA) was made of white plastic with lightly textured arms. The 2 open arms (50×10 cm) had a short 0.9 cm ledge, while the closed arms were the same size as the open but with 30.5 cm high walls. The 10×10 cm central square connected the opposite open and opposite closed arms, thus forming a plus-sign. The maze was elevated 30.5 cm above the floor and a video camera, mounted above the maze, was used to record activity that was later scored by technicians blinded to TSD treatment.

2.3. Procedure

2.3.1. Sleep deprivation

Sixteen rats were designated as experimental and were sleep deprived for 6 h ($N=8$) or 12 h ($N=8$) beginning at light onset (0700 hours). Total sleep deprivation (TSD) was performed by lightly tapping on cages and introducing novel objects to the animals, a method shown to stimulate exploratory behavior and prevent sleep [29]. Animals were watched continuously during TSD. Control animals ($N=16$), located in a separate room, were permitted to sleep either 6 or 12 h ($N=8$ per group) during the same period. Testing for the 6 h-TSD and 6 h-control groups was conducted between 1300 and 1400 hours and between 1900 and 2000 hours for the 12 h-TSD animals and their respective controls. The 12 h light period was extended past 1900 hours on the day of testing to allow for the 1 h needed to test all 12-hour animals. Animals were first tested in the EPM and then immediately sacrificed for neurochemical analysis following either TSD or sleep, depending on group assignment.

2.3.2. Elevated plus maze

Similar to the methods described by Pellow and colleagues, each rat was placed in the center of the maze facing a closed arm and allowed to freely explore for 5 min [30]. The maze was cleaned with a 10% alcohol solution after each subject.

2.3.3. Brain tissue harvesting

Animals were decapitated, brains quickly removed and sliced to 2 mm coronal slices using a brain matrix (ASI, Warren, MI). Anatomically discrete punches (2.1 mm diameter) were obtained for each region of interest in the respective slice. All samples were immediately frozen on dry ice and stored at −80 °C until HR-MAS ^{1}H MRS analysis. Punches were taken from brain regions of the cortico-limbic circuit that are considered important in the regulation of anxiety and fear and included the following: medial prefrontal (infralimbic) cortex (PFC), dorsal hippocampus (HPC), amygdala (AMYG), and medial thalamus (THAL).

2.3.4. HR-MAS ^{1}H MRS

High resolution, magic angle spinning proton magnetic resonance spectroscopy (HR-MAS, ^{1}H MRS), is an ex vivo imaging technique used to obtain rapid, high quality, quantitative, measurements of specific neurochemicals and metabolites in regionally defined samples of excised brain tissue [31–33]. An advantage of HR-MAS, ^{1}H MRS, over traditional magnetic resonance spectroscopy (MRS), is higher signal-to-noise ratios and narrower line widths [34], allowing for more precise quantification of neurochemicals including glutamate. For additional details on HR-MAS, ^{1}H MRS, please refer to our previous investigations [35–38].

Frozen intact tissue samples were weighed (~4 mg), then placed into a Bruker zirconium rotor (2.9 mm diameter, 10 µL capacity; Model 3542, Billerica MA) containing 4 µL of a 0.1 M phosphate-buffered solution (pH = 7.4), and 3-(trimethylsilyl)-1-propane sulfonic acid (1.5 mM, TMS; Sigma, St. Louis, MO). The rotor was then immediately placed into a Bruker 11.7T Avance 500 MR spectrometer where both the rotor and the sample were maintained at 4 °C, spun at a MAS rate of 4200 rpm, and positioned at the magic angle of 54.7° relative to the longitudinal magnetic field (B_0). After a pre-saturation pulse for water suppression, tissue spectra were acquired with a Carr–Purcell–Meiboom–Gill (CPMG) rotor-synchronized pulse sequence (TR = 3500 ms, spectral bandwidth 8 kHz, 16 k complex points, 256 averages, total acquisition time of 15m38s) [33].

2.3.5. Data analysis

Elevated plus maze behavior was scored as follows: The amount of time spent in the closed arms and the number of entries into the closed arms were measured, as well as time in open arms and number of open entries. An entry into an open or closed arm was recorded if all 4 paws crossed into that arm. Total distance traveled was also measured using a pen and paper tracing method.

For neurochemistry, each HR-MAS ^{1}H MRS spectrum was analyzed using the Linear Combination of Model Spectra software program (LCModel) [39]. Using calibration data from solutions with known concentrations, LCModel estimates both the neurochemical concentrations in the sample and the certainty of each concentration measurement (Cramer-Rao bounds). A custom basis set was generated with 28 calibrated phantoms of individual neurochemicals in buffer and conditions identical to those used for sample analysis. TMS resonates at 0.00 ppm and served as the external chemical shift reference. A customized model spectrum was developed in collaboration with Dr. S. Provencher to fit the tissue spectrum and calculate absolute neurochemical concentrations (nmol) with the Bruker 11.7T Avance MR system [39]. Although this method can reliably measure up to 28 neurochemicals and metabolites (Cramer-Rao bounds <10%), we focused our hypothesis on glutamate (Glu), due to its implicated role in fear and anxiety. Absolute concentrations of MR visible neurochemicals were corrected for tissue weight and are expressed as nmol/mg of wet weight (mM).

Because there were no significant differences between the 6- and 12-hour TSD groups with respect to EPM behavior and neurochemistry, they were combined into a single group of total sleep-deprived rats ($N=16$). Similar to TSD, the 6- and 12-hour control rats

demonstrated no behavioral and neurochemical differences and were combined into one, non sleep-deprived, control group ($N=16$). Raw data for the individual 6- and 12-hour groups will be provided to readers upon request. Behavioral data for all TSD and control subjects was available and included in the analyses. Neurochemical data was incomplete due to a lost tissue sample and unreliable quantification (Cramer-Rao bounds>10%) for some samples. Missing neurochemical data were replaced with the conservative approach of a combined TSD and control group mean for the respective regional level of Glu. There was never more than 1 replaced datapoint for either the TSD or control group for any one region. Statistical analysis both with and without the replaced data revealed negligible changes in F and p values and did not change the overall outcome of significant findings reported below.

2.3.6. Statistics

Data analyses were performed using SPSS, Version 16.0 for Windows (SPSS, Inc., Chicago, IL). An overall effect of sleep deprivation on cortico-limbic brain circuit chemistry and behavior was assessed with multivariate F tests for Glu and elevated plus maze variables (i.e. number of open arm entries, percentage of time spent in open arms, and total distance traveled). A significance level of $p\leq 0.05$ was used to assess which dependent variables contributed to an overall effect. The relationship between glutamate levels in the brain and elevated plus maze behavior was assessed with Multiple Regression and Pearson's Correlational Analyses.

3. Results

Behavior: Sleep deprivation had a significant effect on EPM behavior ($F_{2,29}=12.11$, $p<0.01$) and was associated with both reduced fearfulness and increased motor activity. Specifically, the TSD group entered into and spent significantly more time in the open arms of the EPM than the non sleep-deprived control group ($F_{1,31}=9.89$, $p<0.01$; $F_{1,31}=11.03$, $p<0.01$, respectively; see Fig. 1a). Overall activity level, defined by total distance traveled, was also significantly increased in the TSD group compared to the controls ($F_{1,31}=25.05$, $p<0.01$; see Fig. 1b). Time spent in the open arms of the EPM and distance traveled were highly correlated ($r=0.78$, $p<0.01$). Covariate analysis revealed no effect of TSD on the number of entries and time spent in the open arms of the EPM once distance traveled was taken into account.

Neurochemistry: Multivariate analysis revealed a significant main effect of sleep deprivation on glutamate levels in the cortico-limbic brain circuit ($F_{4,27}=2.82$, $p=0.04$). Within this circuit, TSD-induced regional increases in glutamate were also confirmed. Compared to the non sleep-deprived controls, the TSD group had a significantly greater amount of glutamate in the hippocampus ($F_{1,31}=4.22$, $p=0.049$) and medial thalamus ($F_{1,31}=9.09$, $p=0.005$; see Fig. 2).

The data were also analyzed by multiple regression to assess the relationship between sleep deprivation and regional glutamate levels

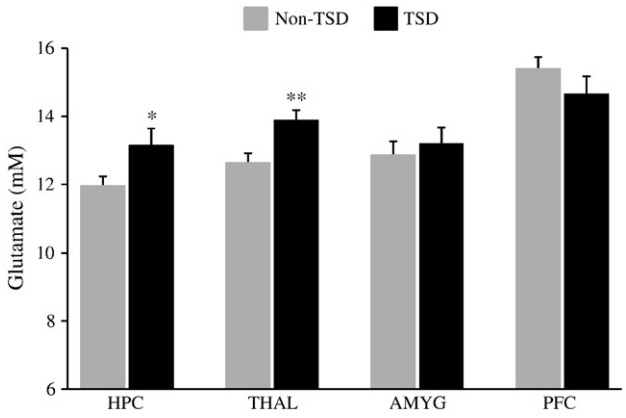

Fig. 2. Regional sleep deprivation-induced increases in glutamate. Glutamate was significantly increased in HPC and THAL in the total sleep deprivation (TSD) compared to the non-TSD rats (* = $p\leq 0.05$, ** = $p\leq 0.01$, HPC—Dorsal Hippocampus, THAL—Medial Thalamus, AMYG—Amygdala, and PFC—Medial Prefrontal Cortex).

(i.e., PFC, HPC, AMYG, and THAL) on risk-taking behavior in the EPM. Although the regression fit was modest ($R^2=41\%$), the overall relationship was significant ($F_{5,31}=3.62$, $p<0.05$). In addition to sleep deprivation ($\beta=0.54$, $t_{26}=3.04$, $p<0.01$), glutamate levels in the medial prefrontal cortex significantly predicted the percentage of time that animals spent in the open arms of the EPM ($\beta=-.32$, $t_{26}=-2.02$, $p\leq 0.05$). No other variables in the model, including glutamate levels in dorsal hippocampus, were significant (see Fig. 3a and b). Pearson's correlation analysis was also utilized in a secondary (exploratory) assessment of the relationship between risk-taking behavior and the ratio of limbic to prefrontal levels of glutamate. An initial analysis of the association between the ratio of glutamate in the dorsal hippocampus to glutamate in the medial prefrontal cortex and the percentage of time spent in the open arms of the maze revealed a positive relationship that reached a trend level of significance ($r=0.28$, $p=0.13$). This analysis, however, exposed an outlier in the ratio measurement of glutamate for one subject. In a subsequent analysis, we replaced the single outlier with a group mean value, revealing a significant positive relationship between the ratio of glutamate in the dorsal hippocampus to glutamate in the medial prefrontal cortex and the percentage of time spent in the open arms of the maze ($r=0.39$, $p<0.05$; see Fig. 3c).

4. Discussion

The present study revealed a sleep deprivation-induced increase in time spent in the open arms of the elevated plus maze (EPM). Traditionally, an increase in open arm behavior in the EPM is thought to be an index of lowered fearfulness, as benzodiazepines are shown to reverse behavioral inhibition in this animal model of fear/anxiety

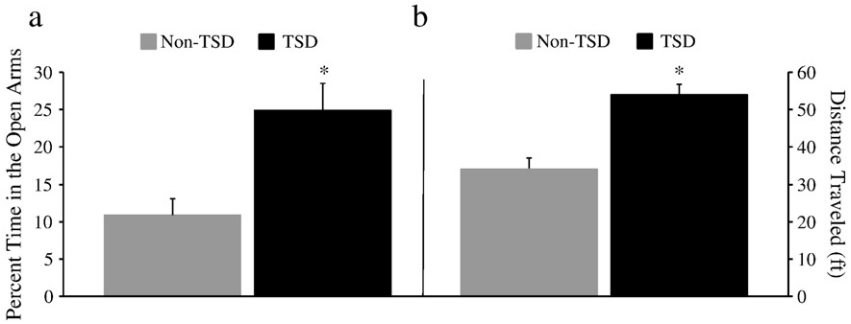

Fig. 1. Elevated plus maze behavior after total sleep deprivation (TSD). The TSD group spent significantly more time in the open arms (a) and traveled a significantly greater distance (b) in the maze compared to the non-TSD group (* = $p<0.01$).

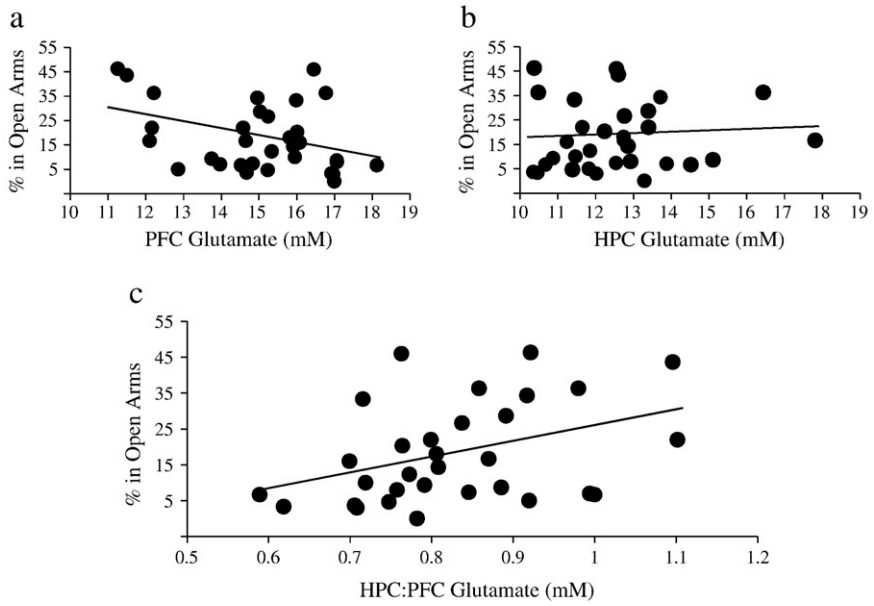

Fig. 3. a–c). Relationship between the concentration of regional glutamate and risk-taking behavior. There was a significant inverse relationship ($r = -0.38$, $p < 0.05$) between PFC glutamate and risk-taking behavior [i.e., percentage of time spent in the open arms of the EPM, (a)], no relationship between HPC glutamate and risk-taking behavior [$r = 0.06$, $p = 0.74$; (b)] and a positive relationship between the ratio of glutamate in dorsal hippocampus to glutamate in medial prefrontal cortical (HPC:PFC glutamate) and risk-taking behavior [$r = 0.39$, $p < 0.05$; (c)].

[4]. More recently, the EPM has also been utilized as a measure of risk assessment and risk-taking behaviors in laboratory animals [40–42]. By classifying entry into and time spent in the open arms of the EPM as risky, our results demonstrate that sleep deprivation was associated with an increase in risk-taking behavior.

The association between sleep deprivation and risk taking is relevant to PTSD because traumatic events (e.g., military combat, motor vehicle accidents, etc.) often occur within the context of sleep restriction [43,44] and individuals with insomnia may be at increased risk for developing PTSD after traumatic experiences [45]. The manner in which sleep deprivation impacts that risk may include the degree to which one would engage in risk-taking behavior and/or situations that may increase the chances of experiencing a traumatic event. Our findings, that sleep deprivation is associated with risk-taking behavior in rats, are consistent with this notion.

In addition to its effect on risk taking, sleep deprivation was associated with an increase in motor activity exemplified by total distance traveled in the EPM. Increased motor activity following sleep deprivation has been reported previously in animal models [13,14,23], as well as depressed humans [20–22,46]. It is possible that the increase in open arm behavior is a byproduct of an increase in general activity/exploratory behavior. The EPM, however, provides a choice between entering the open versus closed arms, so that an animal is never forced to enter or spend any time in the open arms. Even though the TSD rats in the present study were significantly more active than the non-TSD rats, they were free to exclusively explore the closed arms for the duration of test. Despite this fact, they entered into and spent significantly more time in the open arms of the maze, behavior we therefore attribute to increased risk-taking. Clinical studies also show that sleep deprivation alters risk perception and behavior in motor [47], as well as non-motor [24,28] tasks. Extrapolating from the human studies of sleep deprivation-induced effects on non-motor tasks, it is likely that the increase in open arm behaviors is due to increased risk taking, as opposed to the effects of an exclusive increase in motor activity.

Although the sleep deprivation-induced behavioral changes in animal models have been shown to parallel the mood and motor activity effects demonstrated by humans, it is important to point out the methodological differences within both the preclinical and clinical sleep deprivation literature. What we know regarding the behavioral effects of sleep deprivation in humans is largely based on methods using shorter periods (e.g., one full night or second half of the night) of total sleep restriction [20–22,46,48], while the animal studies [14–18,23] have utilized a variety of sleep restriction methods, including prolonged periods of sleep deprivation (i.e., 12 h to several days) and numerous sleep restriction techniques (i.e., sleep fragmentation, selective REM deprivation, or total sleep restriction). Although there are some data [49], much less is known about the effects of the shorter period of sleep deprivation, highlighting the importance of the present study. Surprisingly, we reported no significant differences in both EPM behavior and glutamate levels between the 6-hour and 12-hour TSD groups, suggesting that these behavioral and neurochemical changes take place after very short periods of sleep deprivation. This unexpected finding underscores the need to explore the effects of sleep deprivation over a broader range of sleep deprivation periods to better understand the onset and offset of effects. Additional studies utilizing a range of sleep deprivation periods, therefore are warranted to assess timing of effects on both behavior and neurochemistry.

A main goal of this investigation was to assess post sleep deprivation levels of HR-MAS, ^{1}H MRS assessed glutamate, the most abundant excitatory neurotransmitter in the brain, and determine a potential relationship between sleep deprivation-induced changes in glutamate and behavior in the EPM. Our study revealed a sleep deprivation-induced effect on glutamate levels in the cortico-limbic brain circuit, with significant increases in the dorsal hippocampus and medial thalamus. Although HR-MAS, ^{1}H MRS provides regional, absolute concentrations of glutamate, it cannot distinguish between intracellular and extracellular neurochemical levels and therefore, in the present study, does not inform us as to whether the increases in glutamate were due to increased glutamate neurotransmission and/or alterations in the large metabolic pool of glutamate. Interestingly, sleep deprivation-induced changes in NMDA current amplitude in hippocampal CA-1, in addition to sleep deprivation-induced impairments in long-term potentiation (LTP) and hippocampal-dependent learning and memory have all been well documented [50–53]. Both changes in NMDA-type glutamate receptor number and/or the change in the functional integrity of the NMDA receptors are likely mechanisms for the electrophysiological and behavioral changes demonstrated

after sleep deprivation [54,55]. Although our method of sleep deprivation differed from many of these previous studies, our findings of increased MRS-measured glutamate in dorsal hippocampus is consistent with a compensatory response to a hypothetical sleep deprivation-induced loss of NMDA receptor number and/or function in this brain region.

As reviewed by Bannerman et al. [56], there is an abundance of data in support of the dorsal and ventral hippocampus as anatomically and functionally distinct subregions, with ventral hippocampus having a preferential role in emotion regulation (e.g., fear/anxiety) and dorsal hippocampus related to spatial learning and memory. With our hypotheses regarding the neurobehavioral effects of total sleep deprivation focused primarily on fear and anxiety (i.e., ventral hippocampus-dependent behaviors), glutamate levels in ventral hippocampus are both appropriate and important to assess. Unfortunately we did not obtain tissue samples from this particular subregion and, therefore, it is unknown whether sleep deprivation is associated with HR-MAS ^{1}H MRS-measured glutamatergic changes in the ventral hippocampus.

An increase in glutamate has been previously reported after 12–24 h of paradoxical sleep deprivation in rats [19] and 24-hours of total sleep deprivation in healthy adults [57], the present investigation to our knowledge, however, is the first to assess the relationship between sleep deprivation-induced changes in regional concentrations of glutamate and risk-taking behavior. Our results suggest that the lower the glutamate levels were in medial prefrontal cortex, the higher the percentage of time the animals spent in the open arms of the EPM. The inverse relationship between glutamate in this region and risk-taking behavior is noteworthy because of the strong evidence for the role of the medial prefrontal cortex in successful decision making under risk conditions [58–61]. Although our trend toward a SD-induced decrease in medial prefrontal glutamate was not statistically significant, the inverse relationship, taken together with other independent lines of evidence [62–64], suggests that glutamatergic mechanisms in this brain region may be modulating risk-taking behavior in the EPM. Although our finding suggests a possible role for medial prefrontal glutamate on risk-taking/fear behaviors, it is, of course, possible that other neurotransmitter systems and/or brain regions are important, or even critical, in the mediation of these behaviors.

We also reported a positive relationship between the ratio of glutamate in the dorsal hippocampus to medial prefrontal cortex (HPC: PFC) and risk-taking behavior in the EPM. Of interest, the main focus of most PTSD structural MRI investigation has been the prefrontal cortex and hippocampus, with many of these studies associating PTSD with anterior cingulate gray matter and hippocampal volume reductions [65–71]. Several independent laboratories have also demonstrated an exaggerated limbic (i.e., amygdala) and/or decreased medial prefrontal cortical response to aversive/traumatic stimuli in patients with PTSD [72–76]. Within this context, it is interesting that we demonstrate TSD-induced changes in glutamate in the same brain regions that are consistently associated with PTSD. We speculate, therefore, that glutamatergic mechanisms may be important to risk-taking behaviors that, in humans, could lead to an increased likelihood of traumatic exposures. Future research needs to determine whether these sleep deprivation-induced glutamatergic changes are incidental, or whether they establish a chemical environment in fear-related neurocircuits (i.e. cortico-limbic) that is conducive to the development of PTSD.

References

[1] Bertoglio LJ, Zangrossi H. Involvement of dorsolateral periaqueductal gray N-methyl-D-aspartic acid glutamate receptors in the regulation of risk assessment and inhibitory avoidance behaviors in the rat elevated T-maze. Behav Pharmacol 2006;17:589–96.

[2] Linden AM, Greene SJ, Bergeron M, Schoepp DD. Anxiolytic activity of the MGLU2/3 receptor agonist LY354740 on the elevated plus maze is associated with the suppression of stress-induced c-Fos in the hippocampus and increases in c-Fos induction in several other stress-sensitive brain regions. Neuropsychopharmacology 2004;29:502–13.

[3] Karcz-Kubicha M, Jessa M, Nazar M, Plaznik A, Hartmann S, Parsons CG, et al. Anxiolytic activity of glycine-B antagonists and partial agonists—no relation to intrinsic activity in the patch clamp. Neuropharmacology 1997;36:1355–67.

[4] Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 1986;24:525–9.

[5] Khan S, Liberzon I. Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. Psychopharmacology (Berl) 2004;172:225–9.

[6] Berlant J, van Kammen DP. Open-label topiramate as primary or adjunctive therapy in chronic civilian posttraumatic stress disorder: a preliminary report. J Clin Psychiatry 2002;63:15–20.

[7] Berlant JL. Prospective open-label study of add-on and monotherapy topiramate in civilians with chronic nonhallucinatory posttraumatic stress disorder. BMC Psychiatry 2004;4:24.

[8] Hertzberg MA, Butterfield MI, Feldman ME, Beckham JC, Sutherland SM, Connor KM, et al. A preliminary study of lamotrigine for the treatment of posttraumatic stress disorder. Biol Psychiatry 1999;45:1226–9.

[9] Bremner JD, Mletzko T, Welter S, Quinn S, Williams C, Brummer M, et al. Effects of phenytoin on memory, cognition and brain structure in post-traumatic stress disorder: a pilot study. J Psychopharmacol 2005;19:159–65.

[10] Heresco-Levy U, Kremer I, Javitt DC, Goichman R, Reshef A, Blanaru M, et al. Pilot-controlled trial of D-cycloserine for the treatment of post-traumatic stress disorder. Int J Neuropsychopharmacol 2002;5:301–7.

[11] Baldwin DS, Ajel K. Role of pregabalin in the treatment of generalized anxiety disorder. Neuropsychiatr Dis Treat 2007;3:185–91.

[12] Van Ameringen M, Mancini C, Pipe B, Oakman J, Bennett M. An open trial of topiramate in the treatment of generalized social phobia. J Clin Psychiatry 2004;65: 1674–8.

[13] Suchecki D, Tiba PA, Tufik S. Hormonal and behavioural responses of paradoxical sleep-deprived rats to the elevated plus maze. J Neuroendocrinol 2002;14:549–54.

[14] Pokk P, Zharkovsky A. The effects of flumazenil, Ro 15-4513 and beta-CCM on the behaviour of control and stressed mice in the plus-maze test. J Physiol Pharmacol 1997;48:253–61.

[15] Tartar JL, Ward CP, Cordeira JW, Legare SL, Blanchette AJ, McCarley RW, et al. Experimental sleep fragmentation and sleep deprivation in rats increases exploration in an open field test of anxiety while increasing plasma corticosterone levels. Behav Brain Res 2009;197:450–3.

[16] Meerlo P, Overkamp GJ, Benning MA, Koolhaas JM, Van den Hoofdakker RH. Long-term changes in open field behaviour following a single social defeat in rats can be reversed by sleep deprivation. Physiol Behav 1996;60:115–9.

[17] Hicks RA, Moore JD. REM sleep deprivation diminishes fear in rats. Physiol Behav 1979;22:689–92.

[18] Moore JD, Hayes C, Hicks RA. REM sleep deprivation increases preference for novelty in rats. Physiol Behav 1979;23:975–6.

[19] Bettendorff L, Sallanon-Moulin M, Touret M, Wins P, Margineanu I, Schoffeniels E. Paradoxical sleep deprivation increases the content of glutamate and glutamine in rat cerebral cortex. Sleep 1996;19:65–71.

[20] Post RM, Kotin J, Goodwin FK. Effects of sleep deprivation on mood and central amine metabolism in depressed patients. Arch Gen Psychiatry 1976;33:627–32.

[21] Van Den Burg W, Beersma DG, Bouhuys AL, Van Den Hoofdakker RH. Self-rated arousal concurrent with the antidepressant response to total sleep deprivation of patients with a major depressive disorder: a disinhibition hypothesis. J Sleep Res 1992;1:211–22.

[22] Gerner RH, Post RM, Gillin JC, Bunney Jr WE. Biological and behavioral effects of one night's sleep deprivation in depressed patients and normals. J Psychiatr Res 1979;15:21–40.

[23] Gessa GL, Pani L, Fadda P, Fratta W. Sleep deprivation in the rat: an animal model of mania. Eur Neuropsychopharmacol 1995;5(Suppl):89–93.

[24] Killgore WD, Balkin TJ, Wesensten NJ. Impaired decision making following 49 h of sleep deprivation. J Sleep Res 2006;15:7–13.

[25] Killgore WD, Lipizzi EL, Kamimori GH, Balkin TJ. Caffeine effects on risky decision making after 75 hours of sleep deprivation. Aviat Space Environ Med 2007;78: 957–62.

[26] Killgore WD, Kahn-Greene ET, Lipizzi EL, Newman RA, Kamimori GH, Balkin TJ. Sleep deprivation reduces perceived emotional intelligence and constructive thinking skills. Sleep Med 2008;9:517–26.

[27] McKenna BS, Dicjinson DL, Orff HJ, Drummond SP. The effects of one night of sleep deprivation on known-risk and ambiguous-risk decisions. J Sleep Res 2007;16: 245–52.

[28] Venkatraman V, Chuah YM, Huettel SA, Chee MW. Sleep deprivation elevates expectation of gains and attenuates response to losses following risky decisions. Sleep 2007;30:603–9.

[29] Guan Z, Peng X, Fang J. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. Brain Res 2004;1018:38–47.

[30] Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

[31] Cheng LL, Lean CL, Bogdanova A, Wright Jr SC, Ackerman JL, Brady TJ, et al. Enhanced resolution of proton NMR spectra of malignant lymph nodes using magic-angle spinning. Magn Reson Med 1996;36:653–8.

[32] Tsang TM, Griffin JL, Haselden J, Fish C, Holmes E. Metabolic characterization of distinct neuroanatomical regions in rats by magic angle spinning 1H nuclear magnetic resonance spectroscopy. Magn Reson Med 2005;53:1018–24.

[33] Cheng LL, Ma MJ, Becerra L, Ptak T, Tracey I, Lackner A, et al. Quantitative neuropathology by high resolution magic angle spinning proton magnetic resonance spectroscopy. Proc Natl Acad Sci USA 1997;94:6408–13.

[34] Andrew ER, Eades RG. Removal of dipolar broadening of NMR spectra of solids by spectral rotation. Nature 1802;1959:183.
[35] Bustillo J, Barrow R, Paz R, Tang J, Seraji-Bozorgzad N, Moore GJ, et al. Long-term treatment of rats with haloperidol: lack of an effect on brain N-acetyl aspartate levels. Neuropsychopharmacology 2006;31:751–6.
[36] O'Leary-Moore SK, Galloway MP, McMechan AP, Hannigan JH, Bowen SE. Region-dependent alterations in glutamate and GABA measured by high-resolution magnetic resonance spectroscopy following acute binge inhalation of toluene in juvenile rats. Neurotoxicol Teratol 2007;29:466–75.
[37] O'Leary-Moore SK, McMechan AP, Galloway MP, Hannigan JH. Neonatal alcohol-induced region-dependent changes in rat brain neurochemistry measured by high-resolution magnetic resonance spectroscopy. Alcohol Clin Exp Res 2008;32:1697–707.
[38] Perrine SA, Michaels MS, Ghoddoussi F, Hyde EM, Tancer ME, Galloway MP. Cardiac effects of MDMA on the metabolic profile determined with 1H-magnetic resonance spectroscopy in the rat. NMR Biomed 2009;22:419–25.
[39] Provencher S. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 1993;30:672–9.
[40] Löfgren M, Johansson IM, Meyerson B, Turkmen S, Bäckström T. Withdrawal effects from progesterone and estradiol relate to individual risk-taking and explorative behavior in female rats. Physiol Behav 2009;96:91–7.
[41] Mikics E, Barsy B, Barsvári B, Haller J. Behavioral specificity of non-genomic glucocorticoid effects in rats: effects on risk assessment in the elevated plus-maze and the open-field. Horm Behav 2005;48:152–62.
[42] Griebel G, Rodgers RJ, Perrault G, Sanger DJ. Risk assessment behaviour: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. Pharmacol Biochem Behav 1997;57:817–27.
[43] Sicard B, Jouve E, Blin O. Risk propensity assessment in military special operations. Mil Med 2001;166:871–4.
[44] Hansotia P. Sleep, sleep disorders and motor vehicle crashes. Wis Med J 1997;96: 42–7.
[45] Mellman TA, David D, Kulick-Bell R, Hebding J, Nolan B. Sleep disturbance and its relationship to psychiatric morbidity after Hurricane Andrew. Am J Psychiatry 1995;152:1659–63.
[46] Roy-Byrne PP, Uhde TW, Post RM. Effects of one night's sleep deprivation on mood and behavior in panic disorder. Patients with panic disorder compared with depressed patients and normal controls. Arch Gen Psychiatry 1986;43:895–9.
[47] Lucidi F, Russo PM, Mallia L, Devoto A, Lauriola M, Violani C. Sleep-related car crashes: risk perception and decision-making processes in young drivers. Accid Anal Prev 2006;38:302–9.
[48] Gillin JC. The sleep therapies of depression. Prog Neuropsychopharmacol Biol Psychiatry 1983;7:351–64.
[49] Koehl M, Battle S, Meerlo P. Sex differences in sleep: the response to sleep deprivation and restraint stress in mice. Sleep 2006;29:1224–31.
[50] Campbell IG, Guinan MJ, Horowitz JM. Sleep deprivation impairs long-term potentiation in rat hippocampal slices. J Neurophysiol 2002;88:1073–6.
[51] McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci 2003;23:9687–95.
[52] Kopp C, Longordo F, Nicholson JR, Lüthi A. Insufficient sleep reversibly alters bidirectional synaptic plasticity and NMDA receptor function. J Neurosci 2006;26: 12456–65.
[53] Ravassard P, Pachoud B, Comte JC, Mejia-Perez C, Scoté-Blachon C, Gay N, et al. Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. Sleep 2009;32:227–40.
[54] McDermott CM, Hardy MN, Bazan NG, Magee JC. Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus. J Physiol 2006;570:553–65.
[55] Chen C, Hardy M, Zhang J, LaHoste GJ, Bazan NG. Altered NMDA receptor trafficking contributes to sleep deprivation-induced hippocampal synaptic and cognitive impairments. Biochem Biophys Res Commun 2006;340:435–40.
[56] Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, et al. Regional dissociations within the hippocampus-memory and anxiety. Neurosci Biobehav Rev 2004;28:273–83.
[57] Murck H, Struttmann T, Czisch M, Wetter T, Steiger A, Auer DP. Increase in amino acids in the pons after sleep deprivation: a pilot study using proton magnetic resonance spectroscopy. Neuropsychobiology 2002;45:120–3.
[58] Xue G, Lu Z, Levin IP, Weller JA, Li X, Bechara A. Functional dissociations of risk and reward processing in the medial prefrontal cortex. Cereb Cortex 2009;19:1019–27.
[59] Clark L, Bechara A, Damasio H, Aitken MR, Sahakian BJ, Robbins TW. Differential effects of insular and ventromedial prefrontal cortex lesions on risky decision-making. Brain 2008;131:1311–22.
[60] Bechara A, Damasio AR, Damasio H, Anderson SW. Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 1994;50:7–15.
[61] Bechara A, Tranel D, Damasio H. Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. Brain 2000;123:2189–202.
[62] Coleman Jr LG, Jarskog LF, Moy SS, Crews FT. Deficits in adult prefrontal cortex neurons and behavior following early post-natal NMDA antagonist treatment. Pharmacol Biochem Behav 2009;93:322–30.
[63] Resstel LB, Corrêa FM, Guimarães FS. The expression of contextual fear conditioning involves activation of an NMDA receptor-nitric oxide pathway in the medial prefrontal cortex. Cereb Cortex 2008;18:2027–35.
[64] Lehohla M, Kellaway L, Russell VA. NMDA receptor function in the prefrontal cortex of a rat model for attention-deficit hyperactivity disorder. Metab Brain Dis 2004;19:35–42.
[65] Woodward SH, Kaloupek DG, Streeter CC, Kimble MO, Reiss AL, Eliez S, et al. Hippocampal volume, PTSD, and alcoholism in combat veterans. Am J Psychiatry 2006;163:674–81.
[66] Stein MB, Koverola C, Hanna C, Torchia MG, McClarty B. Hippocampal volume in women victimized by childhood sexual abuse. Psychol Med 1997;27:951–9.
[67] Hedges DW, Allen S, Tate DF, Thatcher GW, Miller MJ, Rice SA, et al. Reduced hippocampal volume in alcohol and substance naïve Vietnam combat veterans with posttraumatic stress disorder. Cogn Behav Neurol 2003;16:219–24.
[68] Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, et al. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 1995;152:973–81.
[69] Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, et al. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse-a preliminary report. Biol Psychiatry 1997;41:23–32.
[70] Yamasue H, Kasai K, Iwanami A, Ohtani T, Yamada H, Abe O, et al. Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. Proc Natl Acad Sci U S A 2003;100: 9039–43.
[71] Villarreal G, Hamilton DA, Petropoulos H, Driscoll I, Rowland LM, Griego JA, et al. Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. Biol Psychiatry 2002;52:119–25.
[72] Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, et al. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 2005;62:273–81.
[73] Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, et al. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol Psychiatry 2000;47:769–76.
[74] Liberzon I, Taylor SF, Amdur R, Jung TD, Chamberlain KR, Minoshima S, et al. Brain activation in PTSD in response to trauma-related stimuli. Biol Psychiatry 1999;45: 817–26.
[75] Britton JC, Phan KL, Taylor SF, Fig LM, Liberzon I. Corticolimbic blood flow in posttraumatic stress disorder during script-driven imagery. Biol Psychiatry 2005;57:832–40.
[76] Bremner JD, Narayan M, Staib LH, Southwick SM, McGlashan T, Charney DS. Neural correlates of memories of childhood sexual abuse in women with and without posttraumatic stress disorder. Am J Psychiatry 1999;156:1787–95.