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Short communication

Antidepressant effects of C-Terminal domain of the heavy chain of tetanus toxin in a rat model of depression

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ABSTRACT

The C-terminal domain of the heavy chain of tetanus toxin (Hc-TeTx) may be of therapeutic potential in motor impairments associated with Parkinson disease (PD). Since depression is a common co-morbid condition with PD, we undertook this study to determine whether Hc-TeTx might also show antidepressant-like properties and whether central brain-derived neurotrophic factor (BDNF) and/or tumor necrosis factor (TNF)-alpha are also affected by it. Adult male Wistar-Kyoto rats, a putative animal model of depression, were treated with various doses of Hc-TeTx (0, 20, 40 and 60 μg/kg, IM) and their performance in the open field locomotor activity (OFLA) as well as in the forced swim test (FST) was evaluated at 24 h, one week and two weeks after the single injection. A separate group of rats were injected with 60 μg/kg Hc-TeTx and sacrificed 24 h later for neurochemical evaluations. Hc-TeTx resulted in a dose-dependent decrease in immobility score after 24 h, whereas OFLA was not affected. Concomitant with the 24 h behavioral effects, the levels of hippocampal and frontal cortical BDNF were significantly increased, whereas the levels of TNF-alpha in both these areas were significantly decreased. The decrease in immobility scores following higher doses of Hc-TeTx were still evident after one week, but not 2 weeks of rest. These results indicate long lasting antidepressant effects of a single Hc-TeTx dose and suggest potential utility of Hc-TeTx in PD-depression co-morbidity.

The toll extracted by clinical depression, characterized by a despondent feeling, loss of interest in pleasurable activities, guilt, worthlessness, and trouble concentrating, is of immense medical concern. This is because the prevalence is relatively high. In US alone, approximately 16 million people or 7% of the adults are afflicted with major depressive disorder, which may also include abnormalities in appetite and sleep and loss of productivity and suicidal ideation. The actual suicide rate, estimated at 1 million worldwide, not only affects the afflicted individual but also the family and friends and at times the entire community [1,2].

Although our understanding of the highly complex neurobiological circuitry of mood regulation remains far from complete, it is known that the symptoms of depression are diverse and vary from patient to patient. In addition, a number of drugs developed over the past 6 decades such as, tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), norepinephrine reuptake inhibitors (NRIs), and selective serotonin reuptake inhibitors (SSRIs) have offered significant relief to at least some of the patients [1].These medications, however, based on biogenic amine theory of depression, which posits that a decrease in these neurotransmitters is the primary cause of the disorder, have several major drawbacks. These include: limited efficacy, delayed onset and various undesirable side effects [3], some of which may be persistent [4]. Hence more rapid onset antidepressants with wider efficacy and lower side effects are urgently needed.

The search for such compounds is facilitated by availability of various models of depression, including the non-induced and treatment-resistant Wistar Kyoto (WKY) rat model. WKY rats, an inbred stain, initially developed as a normotensive control for the spontaneously hypertensive rats [5], were later found to demonstrate exaggerated immobility in the forced swim test (FST), a measure of helplessness or depressive-like behavior [6]. Moreover, it was found that these rats are irresponsive to SSRIs, and hence may be considered as a model of treatment resistant depression [7,8].

Recent elucidation of significant contribution of neurotrophic

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factors and inflammatory processes in mood regulation/dysregulation, has pointed new approaches in development of more effective antidepressants [9–11]. In this regard, several natural and synthetic compounds with anti-inflammatory properties and ability to increase neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF) have been proposed as potential novel antidepressants [9,11,12].

The C-terminal domain of heavy chain of tetanus toxin (Hc-TeTx) is a nontoxic fragment of TeTx with demonstrated capacity to protect against cell death induced by a variety of neurotoxins including methamphetamine [13–18]. Indeed, potential usefulness of peripheral administration of Hc-TeTx in preventing damages in neurodegenerative models such as amyotrophic lateral sclerosis, ischemia, spinal cord injury and more recently in Parkinson's disease (PD) have been reported [19]. Since a strong association between neurodegenerative diseases (e.g. PD) and neuropsychiatric disorders (e.g. depression) in terms of neurobiological substrates as well as drug treatment has been indicated [20], we undertook this study to investigate the potential antidepressant effects of Hc-TeTx in an animal model of depression. Moreover, we hypothesized that any potential antidepressant effect of Hc-TeTx will be associated with an increase in BDNF but a decrease in TNF-alpha in the hippocampus and the frontal cortex, two areas intimately associated with mood regulation [21,22].

Adult male WKY rats (14–15 weeks old) and weighing about 250 g, were obtained from Envigo (previously Harlan Laboratories, Indianapolis, IN). Animals receiving the same treatment were pair-housed through the duration of the experiment in a standard polypropylene shoebox cages (42 × 20.5 × 20 cm) on chip bedding. Animals were subjected to a 1-week acclimatization period upon their arrival, during which they were handled daily to minimize any handling related stress. Throughout the study, with the exception of behavioral tests, animals had free access to food (Harlan Tek Lab) and water. The room was maintained at 24–26°C at 55–66% relative humidity, on a reverse light cycle (lights on 7:00 PM –7:00 AM) to allow convenient behavioral evaluations of the animals during their active period. Acclimatization to reversed dark cycle was done over a one-week period where the light hours were shifted by approximately 2 h daily. All behavioral testing and injections occurred between 8:00 A.M. and 12:00 P.M. during the animal's active phase as described previously [23–25]. All experiments were carried out in accordance with NIH guidelines, as approved by the Institutional Animal Care and Use Committee of the Howard University.

Animals were divided into three groups (n = 6/group) and received intramuscular (i.m.) injection of either saline (control) or 20, 40, or 60 μg/kg dose of Hc-TeTx. The injection was into the *gastrocnemius* muscle. Hc-TeTx fragment was synthesized as described in detail previously [19,26]. The Hc-TeTx solution was prepared by dissolving 1 mg of lyophilized Hc-TeTx in 1 mL of isotonic saline solution, followed by serial dilutions to obtain a final concentration of 20, 40 or 60 μg/100 μL. The volume of injection was 400 μL/kg. Hence each animal received approximately 100 μL of saline or the drug. These doses were chosen based on previous studies where significant motor improvement was observed with such dose range [19].

Approximately 24 h after the last injection, animals were tested in an open-field activity monitoring cage (27 × 27 × 20.3 cm, Med Associates, Inc., St. Albans, VT) for 5 min where ambulatory counts representing the number of infrared beam interruptions were recorded [27]. This behavior was assessed to determine if drug treatment affected general locomotor behavior, which might impinge on forced swim test immobility assessment [25,27].

Immediately following the open field activity test each animal was evaluated for its behavior (immobility) in FST [25, 27]. Briefly, each rat was placed in a Pyrex cylinder pool measuring 17 cm in diameter and 60 cm in height for 5 min. The cylinder was filled with 30 cm water (25 ± 1°C) to ensure that the animals could not touch the bottom of the container with their hind paws or their tails. The FST activity was video recorded for subsequent analysis. The rat was removed after 5 min, dried, and placed in its home cage. A time sampling scoring technique was used whereby the predominant behavior in each 5-s period of the 300- s test was recorded. Inactivity (immobility) and activity (swimming) were distinguished as mutually exclusive behavioral states. Swimming behavior was defined as movement (usually horizontal) throughout the cylinder. Immobility was defined when no additional activity was observed other than that required to keep the rat's head above the water [25,27].

Note: Since behavioral effects were observed a day after a single Hc-TeTx injection, both OFLA and FST were repeated after one week of rest and again after two weeks of rest to determine the lasting effects of the single drug injection on these parameters.

A separate group of rats were treated with the 60 μg/kg dose of Hc-TeTx as this dose had resulted in the highest behavioral (antidepressant) effect. Animals were sacrificed by decapitation, approximately 24 h later to coincide with the time of behavioral observation. No behavioral tests were done in these animals. This was to avoid potential confounding effects of swim test on neurochemical parameters. Brains were quickly removed, frozen on dry ice and stored at -80°C until dissection for BDNF and TNF-alpha measurement. The hippocampus (bilateral) and frontal cortex were dissected as previously described [22].

Western blot was performed as described in detail previously [22,25]. Briefly, homogenate of the dissected hippocampus (bilateral) were made in lysis buffer (10 mM Tris-buffer, 5 mM EDTA, 150 mM NaCl, 0.5% Triton X-100 (v/v) with protease inhibitors (Sigma-Aldrich, St. Louis, MO). The protein concentration in each sample was determined using a BCA protein Assay Kit (Pierce Biotechnology Inc., IL), and equal protein amount (as confirmed by β-actin) was loaded in each immunoblot. The proteins were separated using 12% SDS-PAGE gel and transferred onto a nitrocellulose membrane. The membranes were blocked with a blocking reagent (5% nonfat milk in TBS buffer) for 1/2 h and incubated at 4°C overnight with the primary antibody against BDNF (1:500, Santa Cruz Biotechnology Inc., Santa Cruz, CA) or TNF-alpha (1:500, Santa Cruz Biotechnology). The membranes were washed with TBST (TBS buffer with 1% Tween-20) and blocked with the blocking reagent. Membranes were then incubated for 1 h at room temperature in Goat Anti- Rabbit-HRP conjugated secondary antibody (1:3000 in TBS, Bio-Rad Laboratories, CA). The membranes were then washed in the TBST washing solution and then visualized using enhanced chemiluminescent kits (Bio-Rad Laboratories, CA). The intensity of the protein bands on the gel was quantified using ChemiDoc XRS system (Bio-Rad Laboratories, CA).

Statistical differences between treatment groups were determined by one-way analysis of variance (ANOVA) followed by post-hoc Newman-Keuls Multiple comparison test to determine which groups differed. Significant difference was set a priori at $p < 0.05$. Data were analyzed using Graphpad Prism6 (Graphpad Software, Inc., San Diego, CA, USA).

Single treatment with Hc-TeTx resulted in a dose-dependent decrease in FST immobility when tested 24 h after the injection [$F(328) = 6.38$, $p < 0.01$]. Thus, the 60 μg/kg dose caused the highest decrease (60% $p < 0.01$), the 40 μg/kg (51% $p < 0.01$) and the 20 μg/kg (21% $p < 0.05$) in immobility compared to the control (Fig. 1A). Open field locomotor activity was not altered by any treatment (Fig. 1B), suggesting that the treatment effects of Hc-TeTx on FST were independent of any effects on general locomotion.

Based on these results, we used the highest dose of 60 μg/kg to evaluate the neurochemical changes associated with this behavioral effect.

One week after the last single injection, the effect of 40 and 60 μg/kg doses were still evident on immobility scores [$F(328) = 5.96$, $p < 0.01$]. Hence, with the 40 μg/kg dose, there was 29% decrease in immobility ($p < 0.05$) and with 60 μg/kg dose, there was 39% decrease ($p < 0.01$) (Fig. 2A). After 2 weeks of rest the effect of 40 μg/kg

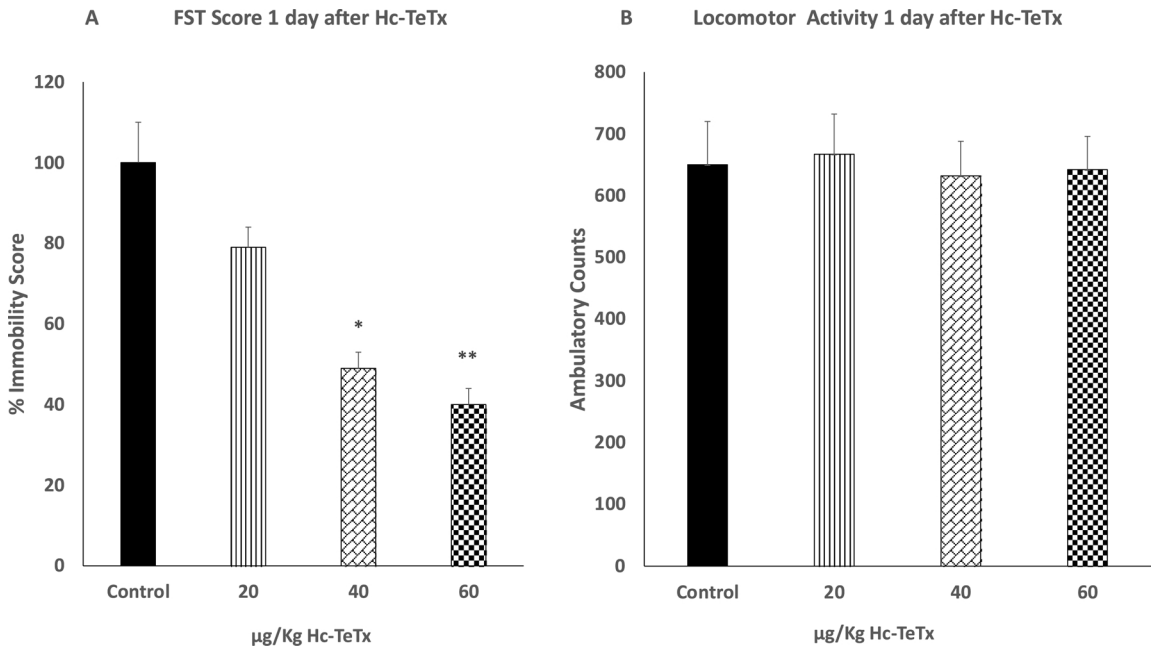

Fig. 1. Effects of various doses of Hc-TeTx on immobility in the forced swim test (1A) and open field locomotor activity (1B) in WKY rats. The animals were tested 24 h after the single i.m. injection. Values are mean ± SEM. N = 6/group. *p < 0.05, **p < 0.01 compared to control.

dose was totally absent (Fig. 2B). Although there was still a 13% decrease in immobility score after the 60 µg/kg dose, this effect was not statistically significant [F(328) = 0.68, p > 0.64].

Western blot analysis showed that acute treatment with the 60 µg/kg dose of Hc-TeTx resulted in increases in BDNF levels in the hippocampal (2.6-fold, p < 0.01) and the frontal cortex (2.1-fold p < 0.01) 24 h after a single administration (Fig. 3). An opposite trend was observed in terms of TNF-alpha levels in both areas. Hence, 60 µg/kg dose of Hc-TeTx resulted in decreases in TNF-alpha levels in the hippocampal (2.5-fold, p < 0.01) and the frontal cortex (5-fold p < 0.01) 24 h after a single administration (Fig. 4).

The results of the current study suggest antidepressant-like effects of an acute dose of Hc-TeTx in an animal model of treatment-resistant depression. This effect was long lasting as the behavioral despair reflected in the immobility scores of the FST was still down one week after the injection. Since potential utility of Hc-TeTx in movement disorders associated with PD has been verified by a number of preclinical studied [19,28] and co-morbidity of depression with PD is also well established [29], it may be concluded that Hc-TeTx would be of specific benefit in such co-morbid condition. This contention is further supported by the findings that neuroprotectants in general, are likely to have antidepressant effects as well [20].

The results also implicate a role for the neurotrophic factor, BDNF and at least one of the pro-inflammatory cytokines, TNF-alpha in

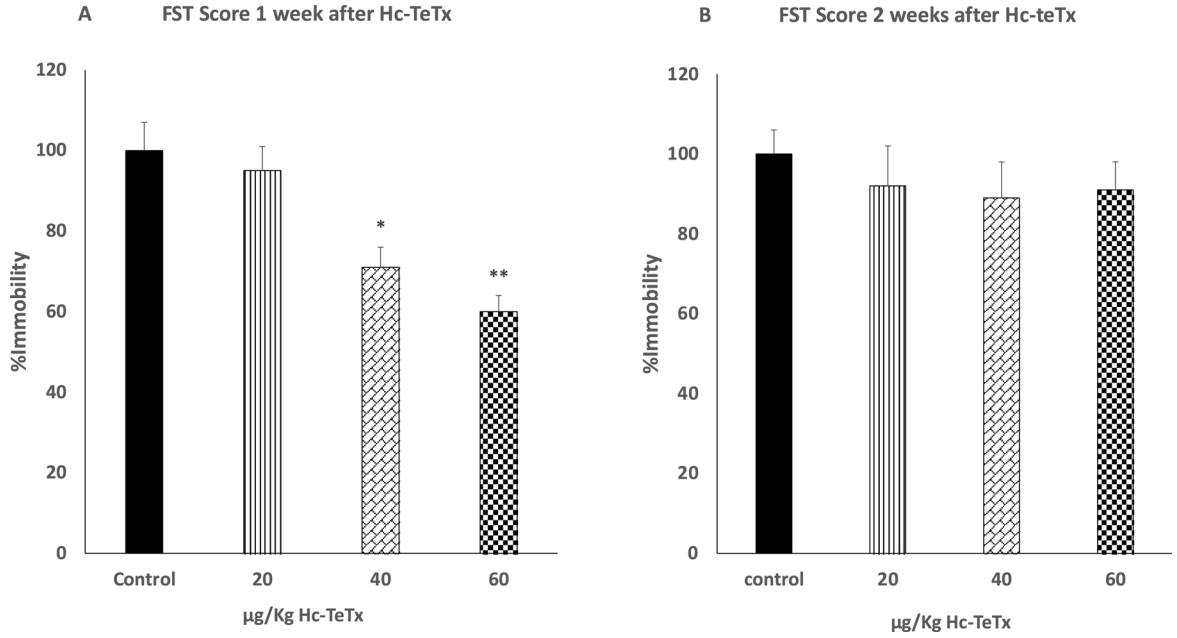

Fig. 2. Effects of various doses of Hc-TeTx on immobility in the forced swim test in WKY rats. The animals were tested one week (2A) and two weeks (2B) after the single i.m. injection.
Values are mean ± SEM. N = 6/group. *p < 0.05, **p < 0.01 compared to control.

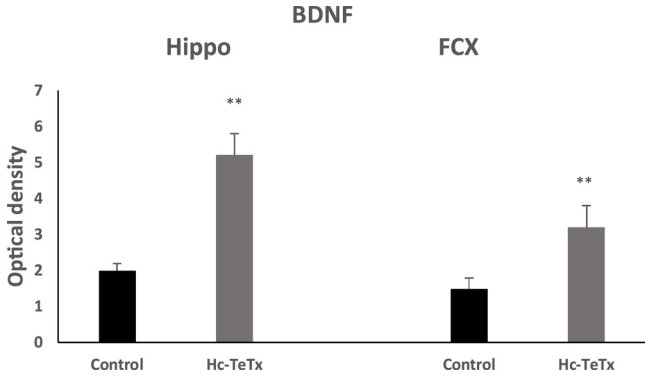

Fig. 3. Effects of Hc-TeTx on BDNF levels in the hippocampus (Hippo) and the frontal cortex (FCX) of WKY rats treated with 60 μg/kg Hc-TeTx. The animals were sacrificed 24 h after the single i.m. injection of Hc-TeTx. Values are mean ± SEM. N = 6/group. **p < 0.01 compared to control.

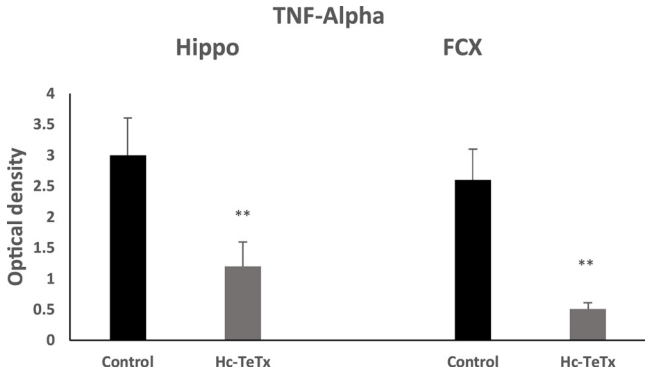

Fig. 4. Effects of Hc-TeTx on TNF-alpha levels in the hippocampus (Hippo) and the frontal cortex (FCX) of WKY rats treated with 60 μg/kg Hc-TeTx. The animals were sacrificed 24 h after the single i.m. injection of Hc-TeTx. Values are mean ± SEM. N = 6/group. **p < 0.01 compared to control.

antidepressant effects of Hc-TeTx. This is due to the fact the levels of the BDNF in both hippocampus and the frontal cortex were elevated by Hc-TeTx, whereas the levels of TNF-alpha were reduced in both these areas a day after the drug injection, concomitant with the observed antidepressant effects.

Although current approved antidepressants are primarily based on the monoaminergic hypothesis, which posits that a decrease in the levels of neurotransmitters such as norepinephrine, dopamine and serotonin (5 H T) in the brain, is responsible for mood dysregulation, the delay in onset of action of such antidepressants and their limited efficacy has shifted the focus to other potential biological substrates. In this regard, a role for neurotrophic factors, particularly hippocampal and also frontal cortical BDNF and more recently, dysregulation of immune system, reflected in elevated levels of pro-inflammatory cytokines such as TNF-alpha have gained substantial traction in the field. Thus, it is now hypothesized that the delay in onset of action of current antidepressants might be due to the delay in elevation of the neurotrophic factors. Whether a delayed anti-inflammatory effect might also be playing a role in late onset of classical antidepressants is not known at this time. It is of relevance to note that es-ketamine, a ketamine analog, which was very recently approved as a quick acting antidepressant, has its proposed mechanism in elevation of hippocampal BDNF [30]. Interestingly, ketamine has also been shown to have anti-inflammatory effects as well [31].

It would be of significant clinical relevance to investigate whether long lasting effects of Hc-TeTx, is solely due to its potential interactions with aforementioned mechanisms or may also involve some effects on biogenic amines. In regard to latter hypothesis, interaction of Hc-TeTx with 5 H T has been noted [32]. Thus, inhibition of both basal and stimulated serotonin uptakes in primary neuronal cultures were demonstrated by Hc-TeTx [32]. In addition, it is necessary to delineate potential sex differences in response to the effects of Hc-TeTx as gender-dependent variation in depression and response to antidepressants is well documented [33,34].

In summary, the results of the current study suggest potential usefulness of Hc-TeTx as a novel intervention in depression-PD co-morbid condition.

Conflict of interest

The authors state no conflict of interest.

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References

[1] M.R. Levinstein, B.A. Samuels, Mechanisms underlying the antidepressant response and treatment resistance, Front. Behav. Neurosci. 8 (2014) 208.
[2] P.E. Greenberg, A.A. Fournier, T. Sisitsky, et al., The economic burden of adults with major depressive disorder in the United States, (2005 and 2010), J. Clin. Psychiatry 76 (2015) 155–162.
[3] A.J. Rush, Targeting treatments for depression: what can our patients tell us? Epidemiol. Psychiatr. Sci. 26 (2017) 37–39.
[4] J. Ben-Sheetrit, D. Aizenberg, A.B. Csoka, et al., Post-SSRI sexual dysfunction: clinical characterization and preliminary assessment of contributory factors and dose-response relationship, J. Clin. Psychopharmacol. 35 (2015) 273–278.
[5] K. Okamoto, K. Aoki, Development of a strain of spontaneously hypertensive rats, Jap Circ J 27 (1963) 282–293.
[6] W.P. Pare, Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats, Physiol. Behav. 55 (1994) 433–439.
[7] C. Lopez-Rubalcava, I. Lucki, Strain-dependent modification of behavior following antidepressant treatment, Prog. Neuropsychopharmacol. Biol. Psychiatry 27 (2003) 7–14.
[8] C.C. Will, E.E. Aird, F. Redei, selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants, Mol. Psychiatry 8 (2003) 925–932.
[9] L.L. Hurley, Y. Tizabi, Neuroinflammation, neurodegeneration, and depression, Neurotox. Res. 23 (2013) 131–144.
[10] O. Kalejaiye, B. Getachew, C.L. Ferguson, et al., Alcohol-induced increases in inflammatory cytokines are attenuated by nicotine in region-selective manner in male rats, J. Drug Alcohol Res. (2017) 6 236036.
[11] C.N. Bodnar, J.M. Morganti, A.D. Bachstetter, Depression following a traumatic brain injury: uncovering cytokine dysregulation as a pathogenic mechanism, Neural Regen. Res. 13 (2018) 1693–1704.
[12] R.S. Duman, BDNF, 5-HT, and anxiety: identification of a critical periadolescent developmental period, Am. J. Psychiatry 174 (2017) 1137–1139.
[13] I. Chaib-Oukadour, C. Gil, J. Rodriguez-Alvarez, et al., Tetanus toxin H(C) fragment reduces neuronal MPP+ toxicity, Mol. Cell. Neurosci. 41 (2009) 297–303.
[14] L. Mendieta, B. Venegas, N. Moreno, et al., The carboxyl-terminal domain of the heavy chain of tetanus toxin prevents dopaminergic degeneration and improves motor behavior in rats with striatal MPP(+)-lesions, Neurosci. Res. 65 (2009) 98–106.
[15] A. Sanchez-Gonzalez, L. Mendieta, V. Palafox, et al., The restorative effect of intramuscular injection of tetanus toxin C-fragment in hemiparkinsonian rats, Neurosci. Res. 84 (2014) 1–9.
[16] L. Mendieta, N. Granado, J. Aguilera, et al., Fragment C domain of tetanus toxin mitigates methamphetamine neurotoxicity and its motor consequences in mice, Int. J. Neuropsychopharmacol. 19 (2016) pyw021.
[17] L. Radenovic, V. Selakovic, S. Olivan, et al., Neuroprotective efficiency of tetanus toxin C fragment in model of global cerebral ischemia in Mongolian gerbils, Brain Res. Bull. 101 (2010) 37–44.
[18] M.C. Sozbilen, M. Ozturk, G. Kaftan, et al., Neuroprotective effects of C-terminal domain of tetanus toxin on rat brain against motorneuron damages after experimental spinal cord injury, Spine, (Phila Pa 1976) 43 (2018) E327–E333.
[19] F. Patricio, I. Parra, I. Martínez, et al., Effectiveness of fragment C domain of tetanus toxin and pramipexole in an animal model of Parkinson's disease, Neurotox. Res. 35 (2019) 699–710.
[20] Y. Tizabi, Duality of antidepressants and neuroprotectants, Neurotox. Res. 30 (2016) 1–13.
[21] J. Jin, S. Maren, Prefrontal-hippocampal interactions in memory and emotion, Front. Syst. Neurosci. 9 (2015) e170.
[22] B. Getachew, S.R. Hauser, A.B. Csoka, et al., Role of cortical alpha-2 adrenoceptors in alcohol withdrawal-induced depression and tricyclic antidepressants, Drug Alcohol Depend. 175 (2017) 133–139.

[23] S.R. Hauser, B. Getachew, R.E. Taylor, et al., Alcohol induced depressive-like behavior is associated with a reduction in hippocampal BDNF, Pharmacol. Biochem. Behav. 100 (2011) 253–258.
[24] Y. Tizabi, B.H. Bhatti, K.F. Manaye, et al., Antidepressant-like effects of low ketamine dose is associated with increased hippocampal AMPA/NMDA receptor density ratio in female Wistar-Kyoto rats, Neuroscience 213 (2012) 72–80.
[25] L. Akinfiresoye, Y. Tizabi, Antidepressant effects of AMPA and ketamine combination: role of hippocampal BDNF, synapsin, and mTOR, Psychopharmacology (Berl.) 230 (2013) 291–298.
[26] M. Herrando-Grabulosa, C. Casas, J. Aguilera, The C-terminal domain of tetanus toxin protects motoneurons against acute excitotoxic damage on spinal cord organotypic cultures, J. Neurochem. 124 (2013) 36–44.
[27] B. Getachew, Y. Tizabi, Both ketamine and NBQX attenuate alcohol-withdrawal induced depression in male rats, J Drug Alc Res 8 (2019) 1–7.
[28] L. Mendieta, E. Bautista, A. Sanchez, et al., The C-terminal domain of the heavy chain of tetanus toxin given by intramuscular injection causes neuroprotection and improves the motor behavior in rats treated with 6-hydroxydopamine, Neurosci. Res. 74 (2012) 156–167.
[29] R.M.J. van der Velden, M.P.G. Broen, M.L. Kuijf, et al., Frequency of mood and anxiety fluctuations in Parkinson's disease patients with motor fluctuations: a systematic review, Mov. Disord. 33 (2018) 1521–1527.
[30] R.S. Duman, Ketamine and rapid-acting antidepressants: a new era in the battle against depression and suicide, F1000Res 7 (2018) pii: F1000 Faculty Rev-659.
[31] S. Loix, M. De Kock, P. Henin, The anti-inflammatory effects of ketamine: state of the art, Acta Anaesthesiol. Belg. 62 (2011) 47–58.
[32] P. Pelliccioni, C. Gil, A. Najib, et al., Tetanus toxin modulates serotonin transport in rat-brain neuronal cultures, J. Mol. Neurosci. 17 (2001) 303–310.
[33] A. Labaka, O. Goñi-Balentziaga, A. Lebeña, et al., Biological sex differences in depression: a systematic review, Biol Res Nursing 20 (2018) 383–392.
[34] J.J. Sramek, M.F. Murphy, N.R. Cutler, Sex differences in the psychopharmacological treatment of depression, Dialog Clin Neurosci 18 (2016) 447–457.