Neuroscience Letters 533 (2013) 34–38

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Predator threat stress promotes long lasting anxiety-like behaviors and modulates synaptophysin and CB1 receptors expression in brain areas associated with PTSD symptoms

Alline Cristina Campos[a,*], Frederico Rogério Ferreira[a,b], Wilson Araujo da Silva Jr.[c,d,e], Francisco Silveira Guimarães[a,b]

[a] Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, 3900 Av. Bandeirantes, Monte Alegre, Ribeirão Preto, São Paulo, Brazil
[b] Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, Brazil
[c] Department of Genetics, School of Medicine of Ribeirão Preto, University of Sao Paulo, 3900 Av. Bandeirantes, Monte Alegre, Ribeirão Preto, São Paulo, Brazil
[d] National Institute of Science and Technology in Stem Cell and Cell Therapy, Brazil
[e] Center for Cell Therapy and Regional Blood Center, Ribeirão Preto, SP, Brazil

HIGHLIGHTS

- We found that predator exposure causes long-lasting anxiogenic effect.
- One week after predator stress SYP expression was found augmented in amygdala.
- Predator stress produces a decrease in CB1 receptor expression in the frontal cortex.

ARTICLE INFO

Article history:
Received 8 August 2012
Received in revised form 16 October 2012
Accepted 3 November 2012

Keywords:
Posttraumatic stress
Predator threat
Animal model
CB1 receptor
Synaptophysin

ABSTRACT

Several studies have suggested that changes in hippocampal, prefrontal cortex and amygdaloid complex function are associated with the main symptoms of Posttraumatic Stress Disorder (PTSD). Predator exposure can mimic some aspects of PSTD such as hyperarousal and chronic anxiety. However, little is known about the neural substrate involved in this model. Synaptophysin (SYP) expression has been used to evaluate synaptic plastic changes while cannabinoids have emerged as a therapeutic target for the treatment of stress- and anxiety-related disorders. The present work evaluated whether the long lasting behavioral effects evoked by predator exposure are associated to long-term changes in the expression of the Cannabinoid receptor 1 (CB1) and the synaptic protein SYP in brain areas related to the genesis of PTSD symptoms (frontal cortex, hippocampus and amygdaloid complex). Male Wistar rats were exposed to a live or a dummy cat and seven days later submitted to the elevated plus maze test. To explore possible neurobiological mechanisms involved in these effects, CB1 receptor and SYP mRNA expression were measured in the hippocampus, frontal cortex and amygdaloid complex. Single predator exposure promoted long-lasting anxiogenic effects. Seven days after predator threat CB1 mRNA expression was down regulated in the frontal cortex and amygdaloid complex while SYP gene was up regulated in the amygdaloid complex. Our results suggested that predator exposure causes long-lasting anxiogenic effects associated with hyperactivation of amygdaloid complex and modulation of CB1 receptor in brain areas related to PTSD symptoms.

1. Introduction

Posttraumatic stress disorder (PTSD) is a debilitating chronic condition that reflects emotional and physiological modifications followed by an initial reaction to a traumatic experience [8]. Patients with PTSD exhibit persistent re-experience of traumatic memories (nightmares, intrusive thoughts) and increased avoidance of trauma-related stimuli (hyper vigilance and hyperarousal) albeit the traumatic event no longer occurs [9].

Even if the neurobiology of this anxiety disorder is not completely understood, several studies associate changes in the hippocampus, prefrontal cortex and amygdala functions with PTSD main symptoms [18,27]. In war veterans or rape victims, for example, the amygdaloid complex seems to be hyperactive while ventral portions of the prefrontal cortex and hippocampus show a volume decrease. These pieces of evidence suggest the involvement of long lasting neuroplastic changes in brain areas associated with emotional memory processing [34,27].

* Corresponding author.
E-mail address: allinecampos@usp.br (A.C. Campos).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.11.016

Animal models of anxiety disorders have been used for decades. However, modeling PTSD in laboratory animals has been a particular challenge since some of its symptoms (nightmares, invasive thoughts) cannot be replicated in rodents [32]. Models based on predator exposure are proposed to mimic some aspects of PSTD such as hyperarousal and chronic anxiety [3,2]. The anxiogenic effects of this procedure are long lasting and persist at least for 3 weeks or more. They are thought to reflect the non-associative sensitized fearfulness manifestations that are observed in PTSD patients [30,31].

The cannabinoid system (ECS) is part of the complex circuitry that regulates anxiety and stress and is a crucial mediator of emotional learning [15,29,1,7,21,38]. In the last decade the ECS has emerged as a therapeutic target for the treatment of stress- and anxiety-related disorders such as post-traumatic stress disorder (PTSD) [29,25]. In humans, potential benefits of the synthetic cannabinoid nabilone were demonstrated in PTSD patients [14], with potential protection against behavioral and endocrine alterations induced by intense stress [15]. Although previous clinical and preclinical evidence pointed to the involvement of ECS on the control of mood and anxiety disorders, the precise mechanisms responsible for these responses remain unclear.

Several studies indicate that the expression of cannabinoid receptor 1 (CB1) receptors is sensitive to stressful situations. For example, animals submitted to a fear conditioning paradigm present CB1 up regulation in the prefrontal cortex [13,28]. In this study the amount of freezing behavior evoked by the fear conditioning test was correlated with CB1 receptor expression in this brain area. CB1 receptors are also modulated in the hippocampus by chronic stress [21,20]. However, no study so far has analyzed the long lasting effects of predator exposure on CB1 receptor expression. The present study, therefore, was aimed at investigating this issue. In addition, changes in the expression of vesicle membrane proteins might contribute to the molecular basis of stress-induced changes in synaptic plasticity and behavior [12,37]. Synaptic vesicle proteins have also been identified as possible factors involved in the pathophysiology of psychiatric disorders such as schizophrenia and depression [37]. Considering the evidence indicating that PTSD is related to plastic changes in these same areas, we also verified if the long lasting behavioral stress responses triggered by predator exposure in rodents could be associated with changes in synaptophysin expression, a protein related to synaptic function [10].

2. Materials and methods

2.1. Subjects

Male Wistar rats (220–250 g) obtained from the colony of the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, were housed in groups of four until the beginning of the experiments. During the behavioral test period they were in single housing. All animals were maintained with free access to food and water in a temperature-controlled room (24 °C) and 12 h light/dark cycle. An adult male cat (3 kg), kept at the animal house of our University Campus with free access to food and water, was used in the present study. A dummy plush cat of approximately the same size of the live cat was used as control. All experiment protocols were carried out according to the Brazilian Society of Neuroscience and Behavior guidelines for care and use of laboratory animals and all efforts were made to minimize animal suffering.

2.2. Apparatus

2.2.1. Predator exposure box

The predator exposure box consisted of a rectangular arena (80 cm × 22 cm × 50 cm) with Plexiglas walls and a metal grid floor [6]. The box was designed to comfortably contain the cat and to provide enough space for measuring the rat presence proximal or distal to the cat compartment. It was divided into two opposed equal compartments (40 cm × 22 cm × 50 cm) separated by a metal grid wall. In the experimental session each rat was placed in the middle of the rat compartment always facing the cat compartment that contained the live or dummy cat. The apparatus was located in a sound attenuated temperature-controlled (25 ± 1 °C) room and the luminosity at the level of the predator box was 60 lux.

2.2.2. Elevated plus maze test (EPM)

The wooden apparatus had two opposite open arms (50 cm × 10 cm) crossed at a right angle by two arms of the same dimensions enclosed by 40-cm-high walls with no roof. The maze was located 50 cm above the floor, and a 1 cm high edge made of Plexiglas surrounded the open arms to prevent falls. The animals were placed inside the maze facing an enclosed arm, and the number of entries and time spent in the open and enclosed arms was automatically recorded by the Anymaze software (Stoelting Co., Wood Dale, USA) for 5 min. The experiment took place in a sound attenuated temperature-controlled (25 ± 1 °C) room and the luminosity at the level of the EPM was 60 lux.

2.3. Behavioral procedures

All rats were daily handled for two days by the experimenter for 5 min and habituated to the predator exposure box for 10 min (pre-exposition). On day 3 the animals were placed in the predator box containing the dummy or live cat during 10 min (predator exposition). The freezing behavior was recorded during the dummy or life cat exposition. To prevent eventual cat smell interference, the cat exposed group was always tested after the group exposed to the dummy cat. Seven days after the arena exposition all animals were tested in the EPM. After each experimental session, the maze was cleaned with a 70% ethanol solution. Immediately after the end of the EPM test the animals were sacrificed under deep urethane (Sigma–Aldrich, St. Louis, MO USA, 5 ml/kg, IP) anesthesia. The brains were immediately removed (5 animals/group), the frontal cortex and the hippocampus were dissected, and punches (1.0 mm diameter) of amygdaloid complex obtained (Fig. 2D). The brain tissues were stored at −70 °C for posterior quantitative PCR analysis.

2.4. Real time quantitative polymerase chain reaction (qPCR)

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA), and cDNA reaction was performed using 1 μg of total RNA at high-capacity cDNA kit (Applied Biosystem, Foster City, CA, USA) according to the manufacturer's instructions. The relative level of mRNA expression of CB1 receptor and the synaptic protein synaptophysin (SYP) genes were evaluated in the StepOne real-time PCR system, using Applied Biosystems real-time master mix with Taqman® gene expression probes (CB1-Assay ID Rn00561409_s1, SYP-Assay IDRn00664697_m1). Total RNA was normalized based on Ct values for GAPDH housekeeping gene (Assay ID Rn01749022_g1). All reactions were duplicated, and fold change was calculated using $2^{-\Delta\Delta Ct}$ method.

2.5. Statistical analysis

Behavioral and mRNA data were analyzed by Student's t test. Correlations were analyzed by Pearson's two-tailed test. The significance level was set at $p \leq 0.05$.

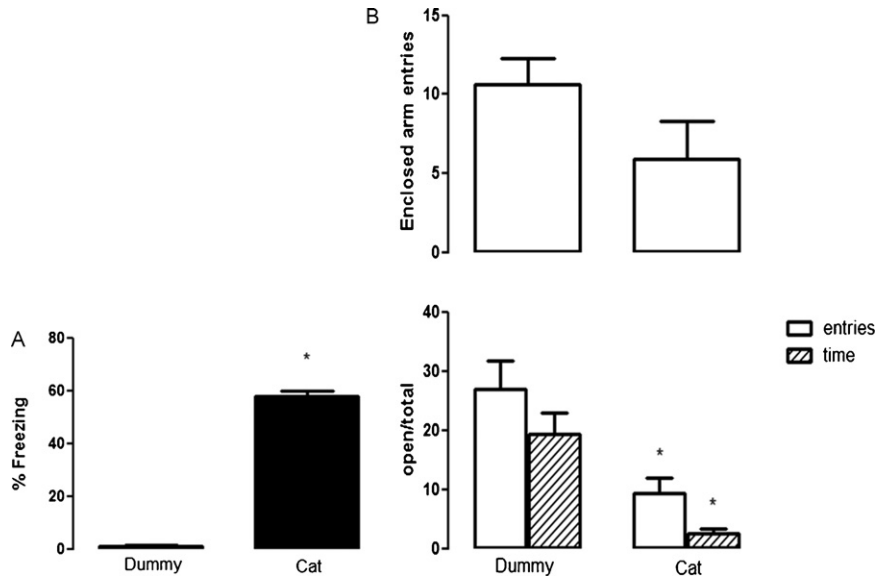

Fig. 1. (A) Percentage of freezing response of rats exposed to a live or a dummy cat. Columns represent mean ± SEM. * indicates a significant difference between groups (Student's *t*-test, $p < 0.001$; $n = 9$ animals/group respectively). (B) Predator exposure induces long-term anxiogenic effects in rats. Animals were submitted to the EPM seven days after a 10 min predator exposure session. Columns represent means ± SEM. In the upper panel the open columns represent the number of enclosed arm entries. In the lower panel open columns represent the percentage of entries onto the open arms while the hatched columns represent the percentage of the time spent in the open arms. * indicates a significant difference from vehicle-live cat exposed rats (Student's *t*-test; $n = 9$ animals/group, respectively).

3. Results

Predator exposure triggered freezing response, reflecting the intense fear reaction evoked by cat exposure (Fig. 1A, $t_{(16)} = 9.7$; $p < 0.001$). One week later rats that had been exposed to the cat presented a significant decrease in the % of entries ($t_{(16)} = 3.3$; $p < 0.01$) and time spent in the open arms ($t_{(16)} = 4.6$; $p < 0.001$) in the EPM. No significant changes in the number of enclosed arm entries in the EPM were observed (Fig. 1B).

The behavioral responses elicited by predator exposure were associated with increased synaptophysin mRNA expression in the amygdaloid complex ($t_{(8)} = 2.34$; $p < 0.05$) (Fig. 2A) and decreased CB1 mRNA expression in the frontal cortex ($t_{(8)} = 5.9$; $p < 0.05$) and the amygdaloid complex, although in this latter structure the

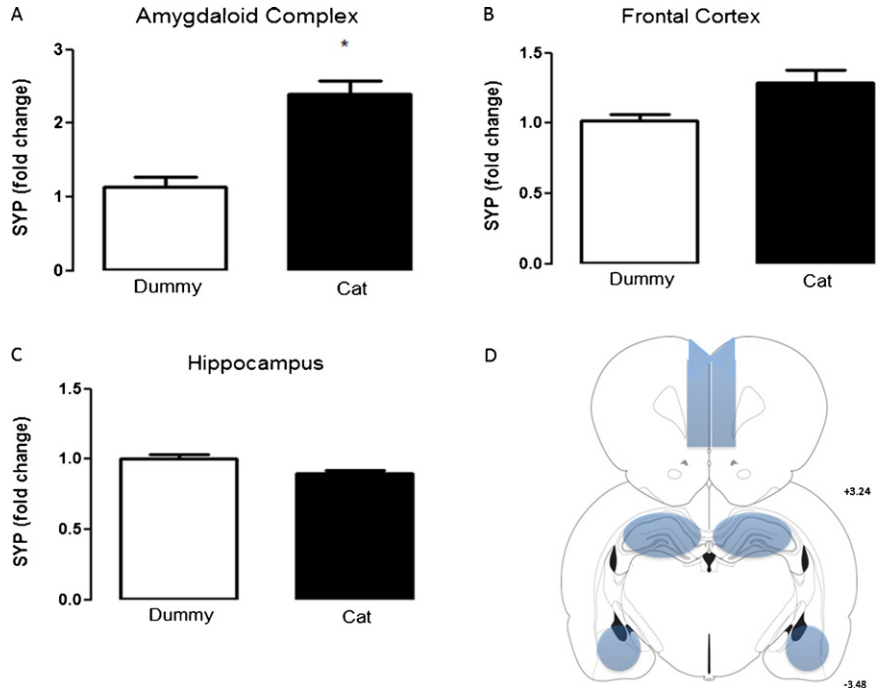

Fig. 2. The long-lasting behavioral effects of predator exposure were associated with changes in Synaptophysin (SYP) mRNA expression in the amygdaloid complex. Rats were sacrificed seven days after predator exposure, 1 h after the EPM test. (A) Amygdaloid complex mRNA fold change; (B) hippocampus mRNA fold change; (C) frontal cortex mRNA fold change; ($2^{-\Delta\Delta Ct}$) mRNA for SYP; Data normalized by GAPDH. Bars represent mean ± SEM of 5 animals/group. * represents a significant general statistical difference from dummy cat exposed rats (Student's *t*-test, $p < 0.05$). (D) Brain sites from where the tissue samples were extracted. The whole frontal cortex (PFC) and the hippocampus were dissected bilaterally. Amygdaloid complex (Amy, bilateral) tissue was obtained by punches (1.0 mm diameter). The numbers refer to the distance from Bregma (based on Paxinos and Watson, 1997).

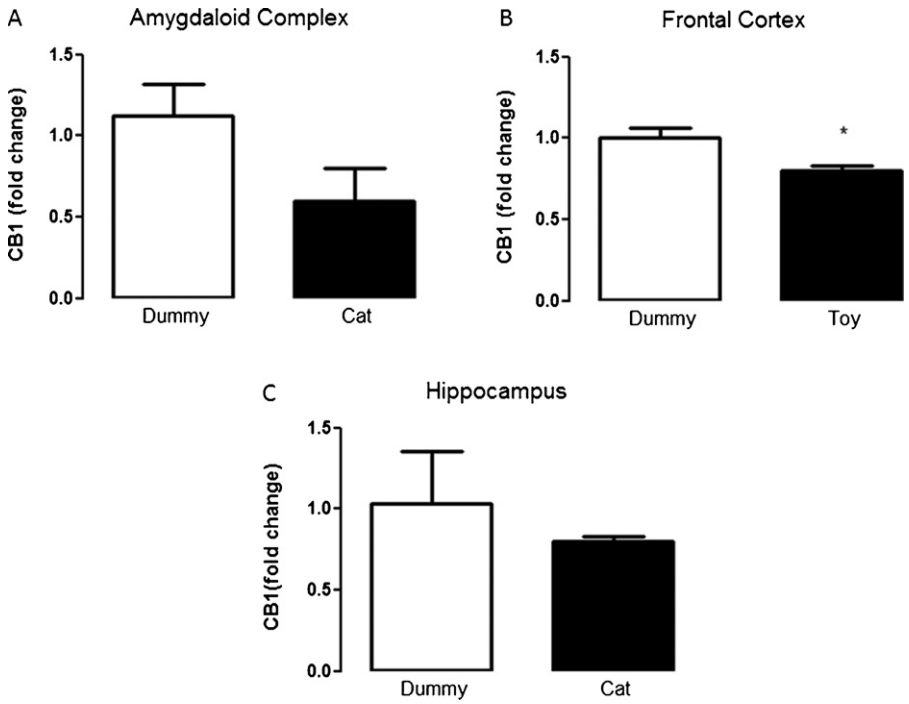

Fig. 3. Long-term anxiogenic effects of a single predator stress session negatively modulate CB1 receptor mRNA expression in the frontal cortex. (A) Amygdaloid complex mRNA fold change; (B) hippocampus mRNA fold change; (C) frontal cortex mRNA fold change; ($2^{-\Delta\Delta Ct}$) mRNA for SYP. Data normalized by GAPDH. * represents a significant general statistical difference from dummy cat exposed rats (Student's *t*-test, $p < 0.05$). Further specifications see Fig. 2 legend.

difference did not reach statistical significance ($t_{(8)} = 1.9$; $p = 0.08$, Fig. 3). No changes in the expression of the SYP and CB1 genes were found in the dorsal hippocampus (Figs. 2 and 3).

Analyses of correlation showed that in amygdaloid complex there is negative correlation between CB1 receptor and SYP expression ($r = 0.66$, $p < 0.01$, Table 1). No similar effects were found in frontal cortex or dorsal hippocampus (Table 1).

4. Discussion

The present work confirmed earlier findings showing that a single predator threat exposure produces long lasting anxiogenic responses in the elevated plus maze [3]. In rodents, exposure to natural predators like cats evokes immediate defensive reactions followed by long-lasting behavioral changes [3,9]. Interestingly, a recent study investigating extinction deficits that have also been associated to PTSD suggested that predator threat stress is a more intense traumatic event than electric foot shocks [16]. Our results also showed that CB1 receptor gene expression decreases in the frontal cortex one week after the traumatic experience (predator threat). Similar trend was found in the amygdaloid complex, but not in the dorsal hippocampus. This latter finding could reflect the apparently contradictory results regarding stress effects on CB1 expression and function within the hippocampus. For example, chronic stress induced a significant reduction in CB1 receptor binding in the dentate gyrus with a parallel increase in the CA3 region [21].

Table 1
Pearson correlations of CB1 and SYP expression in rat brain areas related to PTSD symptoms.

Correlation	*r*-Value	*p*-Value
Frontal cortex	−0.27	ns
Hippocampus	0.11	ns
Amygdaloid complex	−0.66	<0.05

Several studies suggest that the medial prefrontal cortex as a critical site for stress responses. Our findings indicate that cannabinoid-mediated neurotransmission in this region could be involved in these responses. CB1 receptors have a predominant pre-synaptic localization and regulate the release of several neurotransmitters [26,39]. In addition, they are also related to plastic events in the Central Nervous System (CNS), such as LTP, depolarization-induced suppression of inhibition (DSI) and neurogenesis [2,5,24,17]. Because of their high expression in the prefrontal cortex, amygdaloid complex and hippocampus, CB1 receptors are proposed to modify defensive behaviors and consolidation of aversive memories [11,29,28,23] and regulate adaptation to acute or chronic stress [11,19]. In stressful situations, modifications in CB1 mediated neurotransmission can disrupt synaptic information flow. Chronic stress decreases CB1 receptor binding and expression in some regions of the hippocampus [21,20]. Moreover, disruption of endocannabinoid and CB1 receptor signaling promotes activation of the HPA axis, reinforcing the relationship between this system and stressful situations [35,22].

Reinforcing the involvement of CB1 receptor in PTSD, the cannabinoid receptor agonist nabilone was effective in treating resistant nightmares in patients diagnosed with this disorder [14]. Considering that CB1 receptors modulate glutamatergic neurotransmission in the frontal cortex [36], their decreased expression could be facilitating anxiety-like responses by increasing glutamate release in limbic structures (such as the amygdaloid complex). Moreover, based on the negative correlation found between CB1 and SYP gene expression in the amygdaloid complex, we speculate that, if translated in proteins, the long lasting down regulation of CB1 expression promoted by an acute stressful situation could be associated to synaptic remodeling in areas such as the amygdala.

Synaptophysin, also known as p38 synaptic protein, is expressed in almost all neurons in the CNS. It interacts with other synaptic vesicle proteins (such as synaptobrevin) and is related to synaptic function. For this reason, SYP expression has been used to indirectly evaluate the number of synapses [10]. Synaptophysin

mRNA and protein expression in limbic areas can be modulated by stress and antidepressant drugs [12,40,37,33]. Because amygdaloid hyperfunction is a common finding in patients with PTSD diagnosis [34,27], if translated in protein our results with SYP suggest that PTSD models based on predator threat stress mimic not only behavioral but also neurobiological aspects of PTSD. These results could also be related to previous studies suggesting that predator stress induces long-lasting potentiation of excitatory neural transmission in the basolateral amygdala [4].

In conclusion, the present results indicate that predator exposure in rats causes long-lasting (one week) anxiogenic effects associated with changes in SYP and CB1 receptor gene expression in brain areas related to PTSD symptoms. Our results support the idea that CB1 receptor could be a target for anxiety and mood disorders related to stressful life events.

Acknowledgments

We thank Dr. Eleni T. Gomes and José Carlos de Aguiar for their technical support. This research is supported by grants from FAPESP and CNPq. ACC and FRF were FAPESP fellowship recipients.

References

[1] H. Abush, I. Akirav, Short- and long-term cognitive effects of chronic cannabinoids administration in late-adolescence rats, PLoS ONE 7 (2012) 21 e31731.
[2] R.E. Adamec, Transmitter systems involved in neural plasticity underlying increased anxiety and defense – implications for understanding anxiety following traumatic stress, Neuroscience and Biobehavioral Reviews 21 (1997) 755–765.
[3] R.E. Adamec, T. Shallow, Lasting effects on rodent anxiety of a single exposure to a cat, Physiology and Behavior 54 (1993) 101–109.
[4] R. Adamec, M. Toth, J. Haller, J. Halasz, J. Blundell, A comparison of activation patterns of cells in selected prefrontal cortical and amygdala areas of rats which are more or less anxious in response to predator exposure or submersion stress, Physiology and Behavior 105 (3) (2012) 628–638.
[5] T. Aguado, K. Monory, J. Palazuelos, N. Stella, B. Cravatt, B. Lutz, G. Marsicano, Z. Kokaia, M. Guzman, I. Galve-Roperh, The endocannabinoid system drives neural progenitor proliferation, FASEB Journal 19 (2005) 1704.
[6] D.C. Aguiar, F.S. Guimarães, Blockade of NMDA or NO in the dorsal premammillary nucleus attenuates defensive behaviors, Physiology and Behavior 103 (2011) 279–283.
[7] I. Akirav, The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus, Frontiers in Behavioral Neuroscience 5 (2011) 34.
[8] American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 4th ed., text revision, American Psychiatric Association, Washington, DC, 2000.
[9] R.J. Blanchard, D.C. Blanchard, Antipredator defensive behaviors in a visible burrow system, Journal of Comparative Psychology 103 (1989) 70–82.
[10] M.E. Calhoun, M. Jucker, L.J. Martin, G. Thinakaran, D.L. Price, P.R. Mouton, Comparative evaluation of synaptophysin-based methods for quantification of synapses, Journal of Neurocytology 25 (1996) 821–828.
[11] A.C. Campos, F.R. Ferreira, F.S. Guimarães, J.I. Lemos, Facilitation of endocannabinoid effects in the ventral hippocampus modulates anxiety-like behaviors depending on previous stress experience, Neuroscience 167 (2010) 238–246.
[12] E.F. Carvalho-Netto, B. Myers, K. Jones, M.B. Solomon, J.P. Herman, Sex differences in synaptic plasticity in stress-responsive brain regions following chronic variable stress, Physiology and Behavior 104 (2011) 242–247.
[13] K. Choi, T. Le, J. McGuire, G. Xing, L. Zhang, H. Li, C.C. Parker, L.R. Johnson, R.J. Ursano, Expression pattern of the cannabinoid receptor genes in the frontal cortex of mood disorder patients and mice selectively bred for high and low fear, Journal of Psychiatric Research 46 (7) (2012) 882–889.
[14] G.A. Fraser, The use of a synthetic cannabinoid in the management of treatment-resistant nightmares in posttraumatic stress disorder (PTSD), CNS Neuroscience & Therapeutic 15 (2009) 84–88.
[15] E. Ganon-Elazar, I. Akirav, Cannabinoids prevent the development of behavioral and endocrine alterations in a rat model of intense stress, Neuropsychopharmacology 37 (2012) 456–466.
[16] S. Goswami, M. Cascardi, O.E. Rodriguez-Sierra, S. Duvarci, D. Pare, Impact of predatory threat on fear extinction in Lewis rats, Learning & Memory 17 (2010) 494–501.
[17] Y. Hashimotodani, T. Ohno-Shosaku, M. Kano, Endocannabinoids and synaptic function in the CNS, Neuroscientist 13 (2007) 127–137.
[18] C. Heim, C.B. Nemeroff, Neurobiology of posttraumatic stress disorder, CNS Spectrums 14 (2009) 13–24.
[19] M.N. Hill, B.B. Gorzalka, Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test, European Neuropsychopharmacology 15 (2005) 593–599.
[20] M.N. Hill, S. Patel, E.J. Carrier, D.J. Rademacher, B.K. Ormerod, C.J. Hillard, Down-regulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress, Neuropsychopharmacology 30 (3) (2005) 508–515.
[21] M.N. Hill, R.G. Hunter, B.S. McEwen, Chronic stress differentially regulates cannabinoid CB1 receptor binding in distinct hippocampal subfields, European Journal of Pharmacology 614 (2009) 66–69.
[22] M.N. Hill, R.J. McLaughlin, B. Pan, M.L. Fitzgerald, C.J. Roberts, T.T. Lee, I.N. Karatsoreos, K. Mackie, V. Viau, V.M. Pickel, B.S. McEwen, Q.S. Liu, B.B. Gorzalka, C.J. Hillard, Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response, Journal of Neuroscience 31 (2011) 10506–10515.
[23] S.M. Hölter, M. Kallnik, W. Wurst, G. Marsicano, B. Lutz, C.T. Wotjak, Cannabinoid CB1 receptor is dispensable for memory extinction in an appetitively-motivated learning task, European Journal of Pharmacology 510 (2005) 69–74.
[24] W. Jiang, Y. Zhang, L. Xiao, J. Van Cleemput, S.P. Ji, G. Bai, X. Zhang, Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects, Journal of Clinical Investigation 115 (2005) 3104–3116.
[25] S. Kathuria, S. Gaetani, D. Fegley, F. Valino, A. Duranti, A. Tontini, M. Mor, G. Tarzia, G. La Rana, A. Calignano, A. Giustino, M. Tattoli, M. Palmery, V. Cuomo, D. Piomelli, Modulation of anxiety through blockade of anandamide hydrolysis, Nature Medicine 9 (2003) 76–81.
[26] I. Katona, B. Sperlagh, A. Sik, A. Kafalvi, E.S. Vizi, K. Mackie, T.F. Freund, Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons, Journal of Neuroscience 19 (1999) 4544–4558.
[27] M. Koenigs, E.D. Huey, V. Raymont, B. Cheon, J. Solomon, E.M. Wassermann, J. Grafman, Focal brain damage protects against post-traumatic stress disorder in combat veterans, Nature Neuroscience 11 (2008) 232–237.
[28] S.F. Lisboa, D.G. Reis, A.L. da Silva, F.M.A. Correa, F.S. Guimaraes, L.B.M. Resstel, Cannabinoid CB1 receptors in the medial prefrontal cortex modulate the expression of contextual fear conditioning, International Journal of Neuropsychopharmacology 13 (2010) 1163–1173.
[29] G. Marsicano, C.T. Wotjak, S.C. Azad, T. Bisogno, G. Rammes, M.G. Cascio, H. Hermann, J. Tang, C. Hofmann, W. Zieglgansberger, V. Di Marzo, B. Lutz, The endogenous cannabinoid system controls extinction of aversive memories, Nature 418 (2002) 530–534.
[30] R.K. Pitman, Overview of biological themes in PTSD, Annals of the New York Academy of Sciences 821 (1997) 1–9.
[31] R.K. Pitman, Will reconsolidation blockade offer a novel treatment for post-traumatic stress disorder? Frontiers in Behavioral Neuroscience 5 (2011) 11.
[32] R.K. Pitman, S.P. Orr, A.Y. Shalev, Once bitten, twice shy: beyond the conditioning model of PTSD, Biological Psychiatry 33 (1993) 145–146.
[33] S. Rapp, M. Baader, M. Hu, C. Jennen-Steinmetz, F.A. Henn, J. Thome, Differential regulation of synaptic vesicle proteins by antidepressant drugs, Pharmacogenomics Journal 4 (2004) 110–113.
[34] L.M. Shin, S.L. Rauch, R.K. Pitman, Amygdala, medial prefrontal cortex, and hippocampal function in PTSD, Annals of the New York Academy of Sciences 1071 (2006) 67–79.
[35] M.A. Steiner, C.T. Wotjak, Role of the endocannabinoid system in regulation of the hypothalamic-pituitary-adrenocortical axis, Progress in Brain Research 170 (2008) 397–432.
[36] K.A. Takahashi, P.E. Castillo, The CB1 cannabinoid receptor mediates glutamatergic synaptic suppression in the hippocampus, Neuroscience 3 (139) (2006) 795–802.
[37] J. Thome, B. Pesold, M. Baader, M. Hu, J.C. Gewirtz, R.S. Duman, F.A. Henn, Stress differentially regulates synaptophysin and synaptotagmin expression in hippocampus, Biological Psychiatry 50 (2001) 809–812.
[38] M.P. Viveros, E.M. Marco, S.E. File, Endocannabinoid system and stress and anxiety responses, Pharmacology Biochemistry and Behavior 81 (2005) 331–342.
[39] R.I. Wilson, G. Kunos, R.A. Nicoll, Presynaptic specificity of endocannabinoid signaling in the hippocampus, Neuron 16 (2001) 453–462.
[40] H. Xu, J. He, J.S. Richardson, X.M. Li, The response of synaptophysin and microtubule-associated protein 1 to restraint stress in rat hippocampus and its modulation by venlafaxine, Journal of Neurochemistry 91 (2004) 1380–1388.