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Brain Research

Research Report

TMT predator odor activated neural circuit in C57BL/6J mice indicates TMT-stress as a suitable model for uncontrollable intense stress

K. Janitzky[a,b,*], W. D'Hanis[c], A. Kröber[c], H. Schwegler[c]

[a]University Department of Neurology, Otto-von-Guericke University, D-39120 Magdeburg, Germany
[b]Leibniz Institute for Neurobiology, D-39118 Magdeburg, Germany
[c]Institute of Anatomy, University of Magdeburg, D-39120 Magdeburg, Germany

ARTICLE INFO

Article history:
Accepted 11 December 2014
Available online 19 December 2014

Keywords:
Trimethylthiazoline
Predator odor
Fos-ir
BNST
Locus coeruleus
PTSD

ABSTRACT

Intense stressful events can result in chronic disorders such as posttraumatic stress disorder (PTSD). In vulnerable individuals, a single aversive experience can be sufficient to cause long-lasting behavioral changes. Candidate brain regions implicated in stress-related psychopathology are the amygdala, the bed nucleus of the stria terminalis (BNST), and the hypothalamic pituitary adrenal (HPA) axis. In rodents exposure to 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), an ethologically relevant stressor, has been shown to induce intense stress and innate anxiety responses. To study dispositions for the development of maladaptive stress responses, mice models are required. Therefore C57BL/6J mice were exposed to TMT and Fos expression was studied in key brain regions implicated in stress responses and anxiety-like behavior. Our results show TMT-induced activation of a distinct neural circuit involving the BNST, the lateral septum (LS), the paraventricular nucleus of the hypothalamus (PVN), the periaqueductal gray (PAG) and the locus coeruleus (LC). Anatomical interconnection of the BNST with all these regions could point to an important modulatory role of this nucleus. Since, the BNST gets direct input from the olfactory bulbs and projects to the PVN and PAG and is therefore well positioned to modulate behavioral and endocrine stress responses to TMT. Hence, we suggest that TMT exposure is suitable to investigate uncontrollable stress responses in mice which exhibit similarities to maladaptive stress responses underlying PTSD in humans.

1. Introduction

In response to a traumatic stressful event, vulnerable individuals can develop posttraumatic stress disorder (PTSD) characterized by intense anxiety and fear over time. The neuronal mechanisms underlying individual differences in the vulnerability of humans to develop PTSD are not fully understood. However, research in the last years has begun to

*Corresponding author at: University Department of Neurology, Otto-von-Guericke University, Leipziger Str. 44, D-39120 Magdeburg and Leibniz Institute for Neurobiology, D-39118 Magdeburg, Germany.
E-mail address: kathrin.janitzky@med.ovgu.de (K. Janitzky).

http://dx.doi.org/10.1016/j.brainres.2014.12.030
0006-8993/

identify some essential brain structures which seem to be involved in PTSD-related maladaptive stress responses such as the amygdala, the bed nucleus of the stria terminalis (BNST), the hypothalamic pituitary adrenal (HPA) axis and the locus coeruleus (LC) (Conrad et al., 2011; Lebow et al., 2012; Olson et al., 2011; Radley et al., 2008). Animal models offer the opportunity to study neuronal activation using Fos expression in response to intense stressors (Yehuda et al., 2006). PTSD has been modeled in rodents by exposure to a predator because predator exposure induced long-lasting behavioral changes similar to severe traumatic events leading to PTSD in humans (Campos et al., 2013). Especially predator odors, which induce long-lasting behavioral and physiological effects, are useful tools to elicit innate fear in mice with ethological relevance (Apfelbach et al., 2005; Fendt et al., 2003; Figueiredo et al., 2003; Takahashi, 2014). Hence, predator odors are particularly suited to induce stress in rodents, as rodents are very sensitive to olfactory cues, and odors have been shown to influence nearly every major class of rodent behavior (Morrow et al., 2000). Exposure to 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), a synthetically derived component of fox feces (Vernet-Maury, 1980), can elicit innate fear behavior associated with endocrine responses (Fendt et al., 2005; Janitzky et al., 2009; Laska et al., 2005; Morrow et al., 2000; Müller and Fendt, 2006; Myers and Rinaman, 2005; Vernet-Maury, 1980). Furthermore, TMT offers several advantages as compared to other predator or predator odor stimuli. For one thing, as a synthetic compound, TMT can be presented with high precision and reproducibility as it is not influenced by factors like the diet of a predator, which could potentially affect the efficiency of the odor. For another thing, in contrast to the exposure to a living cat, TMT exposure is a relatively non-intrusive method for stressing animals (Day et al., 2004; Endres and Fendt, 2008; Figueiredo et al., 2003; Laska et al., 2005; Morrow et al., 2000).

Hence, we used TMT exposure as stressor to uncover brain regions involved in maladaptive stress responses in C57BL/6J mice. Therefore, Fos expression as a marker for neuronal activation was quantified in brain regions previously described to be implicated in fear, anxiety and stress-related behavior, namely the bed nucleus of the stria terminalis (BNST), the medial nucleus of the amygdala (MeA), the central nucleus of the amygdala (CeA), the lateral septum (LS), the paraventricular nucleus of the hypothalamus (PVN), the periaqueductal grey (PAG) and the locus coeruleus (LC) (Campeau et al., 1997; Day et al., 2004; Dielenberg et al., 2001; Fendt et al., 2005; Figueiredo et al., 2003; Hebb et al., 2004; Matsukawa et al., 2011; Murakami et al., 2012; McGregor et al., 2004; Radulovic et al., 1998; Staples et al., 2008). Since prior studies in rats have shown inconsistent results with respect to neuronal activation patterns after TMT exposure (Asok et al., 2013; Day et al., 2004; Funk and Amir, 2000; Staples et al., 2008), we believe that a more detailed investigation of TMT activated neural circuits in wild-type mice is required before TMT exposure can be meaningfully applied as a model for uncontrollable stress in transgenic mice. Therefore, in our study we used C57BL/6J mice, a mouse strain that is often used as the background strain for transgenic mice.

2. Results

A total of ten brain regions were quantified using variable numbers of slices (CeA 7 slices, MeAad 4 slices, MeApd 2 slices, MeAav 4 slices, MeApv 2 slices, PVN 4 slices, BNST 7 slices, LC 2 slices, LS 9 slices, PAG 16 slices). Fig. 1 shows the mean number of Fos-positive cells within each area (one hemisphere) for the three experimental conditions. The ANOVA shows an overall group effect in the lateral septum (LS) ($F_{(2,18)}$ 31,224, $p<0.001$), the anterodorsal part of the medial amygdalar nucleus (MeAad) ($F_{(2,18)}$ 16,238, $p<0.001$), the anteroventral part of the medial amygdalar nucleus (MeAav) ($F_{(2,18)}$ 10,676, $p=0.001$), the posterodorsal part of the medial amygdalar nucleus (MeApd) ($F_{(2,18)}$ 11,059, $p=0.001$), the posteroventral part of the medial amygdalar nucleus (MeApv) ($F_{(2,18)}$ 5,407, $p=0.014$), the central amygdala (CeA) ($F_{(2,18)}$ 5,062, $p=0.018$), the bed nucleus of the stria terminalis (BNST) ($F_{(2,18)}$ 47,917, $p<0.001$), the paraventricular nucleus of the hypothalamus (PVN) ($F_{(2,18)}$ 25,364, $p<0.001$), the periaqueductal grey (PAG) ($F_{(2,18)}$ 51,373, $p<0.001$) and the locus coeruleus (LC) ($F_{(2,17)}$ 22,042, $p<0.001$).

Post-hoc tests indicate that TMT-exposed mice show significantly more Fos expression as compared to homecage controls in the LS ($p<0.001$), the MeAad ($p<0.001$), the MeAav ($p=0.001$), the MeApd ($p=0.001$), the MeApv ($p=0.015$), the CeA ($p=0.019$), the BNST ($p<0.001$), the PVN ($p<0.001$), the PAG ($p<0.001$) and the LC ($p<0.001$).

Diethylphthalate (DEP) that was used as a solvent to dilute TMT in prior studies (Laska et al., 2005) was used it as control odor in the present study. Compared to DEP-exposed controls, TMT-exposed mice show significantly increased Fos expression in slices of the BNST ($p<0.001$, Fig. 1G), the PVN ($p=0.002$, Fig. 1F), the LS ($p=0.002$, Fig. 1I), the PAG ($p<0.001$, Fig. 1J) and the LC ($p=0.003$, Fig. 1H). Representative slices of regions with significant TMT-induced Fos induction are shown in Fig. 2. No significant differences in Fos expression were found between TMT- and DEP-exposed mice in the MeA ($p>0.05$) and the CeA ($p>0.05$).

Compared to homecage mice, DEP-exposed control mice showed significantly increased Fos expression in slices of the LS ($p=0.013$), the MeAad, ($p<0.012$), the BNST ($p<0.017$) and the PAG ($p<0.01$).

3. Discussion

Our results show TMT-related activation of a distinct brain circuit including the bed nucleus of the stria terminalis (BNST), the lateral septum (LS), the paraventricular nucleus of the hypothalamus (PVN), the locus coeruleus (LC) and the periaqueductal grey (PAG) in mice. Among these structures, the BNST is particularly well positioned to control predator odor-induced unconditioned behavioral and endocrine stress responses, due to its prominent anatomical connections with limbic, hypothalamic and brainstem structures (Endres and Fendt, 2008; Fendt et al., 2003, 2005; Takahashi et al., 2005). Furthermore, given that the BNST receives direct input from the main olfactory bulb (MOB) (Day et al., 2004; Funk and Amir, 2000; Kobayakawa et al., 2007; McGregor et al., 2004; Staples et al., 2008) as well as from the anterior piriform