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Research report

# Habitat odor can alleviate innate stress responses in mice

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ABSTRACT

Predatory odors, which can induce innate fear and stress responses in prey species, are frequently used in the development of animal models for several psychiatric diseases including post-traumatic stress disorder (PTSD) following a life-threatening event. We have previously shown that odors can be divided into at least three types; odors that act as (1) innate stressors, (2) as innate relaxants, or (3) have no innate effects on stress responses. Here, we attempted to verify whether an artificial odor, which had no innate effect on predatory odor-induced stress, could alleviate stress if experienced in early life as a habitat odor. In the current study, we demonstrated that the innate responses were changed to counteract stress following a postnatal experience. Moreover, we suggest that inhibitory circuits involved in stress-related neuronal networks and the concentrations of norepinephrine in the hippocampus may be crucial in alleviating stress induced by the predatory odor. Overall, these findings may be important for understanding the mechanisms involved in differential odor responses and also for the development of pharmacotherapeutic interventions that can alleviate stress in illnesses like PTSD.



## 1. Introduction

Alleviating stress is a basic requirement for human welfare, as well as the welfare of laboratory animals. It is known that stress, especially an exposure to a life-threatening event, can develop into chronic disorders such as post-traumatic stress disorder (PTSD). Although definitive biomarkers for the development of PTSD remain elusive, recent studies have shown

Abbreviations: 5-HT, serotonin; ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; APC, anterior piriform cortex; BLA, basolateral complex of amygdala; BST, bed nucleus of stria terminalis; DHBA, 3,4-dihydroxybenzylamine; EDTA, ethylenediaminetetraaceticacid; hinokitiol, 2-hydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one; HPA, hypothalamo–pituitary–adrenal gland; HPLC-ECD, high performance liquid chromatography with an electrochemical detection system; LC, locus coeruleus; NE, norepinephrine; OB, olfactory bulb; PFC, prefrontal cortex; PTSD, post-traumatic stress disorder; TMT, 2,5-Dihydro-2,4,5-trimethylthiazoline

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that the hypothalamic–pituitary–adrenal (HPA) axis is involved (Liberzon et al., 1999; Söndergaard et al., 2004; Oosthuizen et al., 2005; Wilson et al., 2013; Zoladz and Diamond, 2013) and that the modulatory effects of some neurotransmitters are implicated in the disorder (Arora et al., 1993; Geracioti et al., 2001; Krystal and Neumeister, 2009; Southwick and Charney, 2012). In PTSD animal models, lower serotonin (5-HT) and elevated norepinephrine (NE) levels have recently been shown in some brain regions (Wilson et al., 2014). Hence, the regulation of such neurotransmitters has been the primary target of therapeutic intervention for PTSD treatment (Krystal and Neumeister, 2009).

Odors from predatory animals are known to induce the innate stress response in prey animals. Exposure to a predator or predatory odor is used to create animal models of some neuropsychological disorders including anxiety, phobia and PTSD (Rosen et al., 2008; Staples, 2010; Zoladz et al., 2012). A synthetic compound mimicking the anal gland secretions of a red fox, 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), is one of the most commonly used odors to induce a fear response and stress-like behavior in rodents (Vernet-Maury et al., 1984; Apfelbach et al., 2005; Fendt et al., 2005; Takahashi et al., 2008; Takahashi, 2014). In addition, several reports have shown that neonatal or pre-puberty exposure to TMT can exert a sex-related differential effect on fear-related behaviors (Toledo-Rodriguez and Sandi, 2007) and can decrease avoidance, immobility, and freezing during adulthood (Hacquemand et al., 2010). The bed nucleus of the stria terminalis (BST) is thought to be involved in these odor-induced stress-like responses (Fendt et al., 2003; Kobayakawa et al., 2007; Takahashi, 2014; Janitzky et al., 2015). Furthermore, TMT can increase the efflux of monoamines in the mouse brain (Hayley et al., 2001; Smith et al., 2006) and can activate the locus coeruleus (LC) from which noradrenergic fibers innervate the cerebral cortex, amygdala and hippocampus (Day et al., 2004; Curtis et al., 2012; Janitzky et al., 2015).

TMT-induced stress-like responses in mice can be allayed by simultaneous presentation with rose odor (from Bulgarian rose oil) or hinokitiol odor (2-hydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one; a chemical constituent of woody oil from Thujopsis heartwood) but not in combination with caraway odor (S (+)-carvone) (Matsukawa et al., 2011; Murakami et al., 2012). These studies have shown that there are at least three types of odor in nature: (1) odors that can innately induce stress-like behavior, such as predator odors like TMT; (2) odors that can innately alleviate predatory odor-induced stress-related activities, such as rose and hinokitiol odor and (3) odors that have no effect, such as caraway odor. Hence, we hypothesized that postnatal experiences may have a modifying effect on the innate responses, leading to changes that may cause individual differences in odor response in adulthood.

In the current study, we attempted to validate this hypothesis. In particular, a non-effective odor experienced as a habitat odor during the early postnatal period can alleviate TMT-induced stress in adulthood. To assess stress levels, we measured plasma concentrations of adrenocorticotropic hormone (ACTH), a biomarker of the HPA axis. Neuronal activation in the anterior piriform cortex (APC) and the BST was measured by assessing c-fos expression levels. We also measured NE concentrations in the prefrontal cortex (PFC) and hippocampus. Previous studies of both these regions have shown an increase in NE levels following TMT exposure (Wilson et al., 2014).

## 2. Results

### 2.1. *Shredded newspaper odor could alleviate TMT-induced stress*

We used the artificial odor of shredded newspaper, which we anticipated to be an innate non-effective odor. First, we assessed whether the newspaper odor was non-effective by measuring plasma ACTH concentrations. Animals housed with standard laboratory bedding were used in this assessment. Exposure to TMT alone (T) or TMT in combination with newspaper odor (TN) significantly increased plasma ACTH levels compared with the odorless control (DDW) (one-way ANOVA: $F$ (2, 15)=8.237, $P<0.01$). Hence, the shredded newspaper odor was determined to be an innate non-effective odor for mice.

In further experiments, we used shredded newspaper as bedding for mice during the 3-week lactational period and no bedding for 3 subsequent weeks. Exposure to TMT alone (T) significantly increased plasma ACTH concentrations but no changes were found when TMT was presented in combination with newspaper odor (TN) (one-way ANOVA: $F$ (2, 15)= 29.15, $P<0.001$). This result shows that even the innate non-effective odor of shredded newspaper could alleviate the TMT-induced stress response if experienced as a habitat odor in early life (two-way ANOVA: bedding in early life, $P<0.05$, odor, $P<0.05$, interaction, $P<0.05$) (Fig. 1).

### 2.2. *Changes in the density of activated neurons in the brain*

We previously reported that there are at least two distinct mechanisms involved in allaying TMT-induced stress-related activities. First, counteracting mechanisms that can directly suppress the neuronal activities in related brain regions, as shown in combination with rose odor and second, mechanisms in which the selective responses to TMT become indistinguishable, as shown using hinokitiol odor (Matsukawa et al., 2011; Murakami et al., 2012). We next assessed which mechanisms were involved in alleviating TMT-induced stress during simultaneous presentations of habitat odor, by counting c-fos expressing neurons in the APC and the BST. Activated neurons following the presentation of each odor, in the dorsal or ventrorostral APC (APCd and APCvr, respectively), are shown in Fig. 2. Although there was a significant increase in activation between each odor and its odorless control (DDW) in both APCd (one-way ANOVA: $F$ (4, 55)=18.45, $P<0.001$) and APCvr (one-way ANOVA: $F$ (4, 55)=18.51, $P<0.001$), there were no significant differences between early bedding vs odor presentation in the APCd (two-way ANOVA: bedding in early life, $P=0.873$, odor, $P=0.633$, interaction, $P=0.601$) (Fig. 2a). In contrast, significantly fewer neurons were activated following presentation of TMT with newspaper odor (TN), for animals housed with newspaper bedding during the lactational period (gray bar) in the APCvr (two-way ANOVA: bedding in early life,