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Maternal programming of sex-specific responses to predator odor stress in adult rats

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ABSTRACT

Prenatal stress mediated through the mother can lead to long-term adaptations in stress-related phenotypes in offspring. This study tested the long-lasting effect of prenatal exposure to predator odor, an ethologically relevant and psychogenic stressor, in the second half of pregnancy. As adults, the offspring of predator odor-exposed mothers showed increased anxiety-like behaviors in commonly used laboratory tasks assessing novelty-induced anxiety, increased defensive behavior in males and increased ACTH stress reactivity in females in response to predator odor. Female offspring from predator odor-exposed dams showed increased transcript abundance of glucocorticoid receptor (*NR3C1*) on the day of birth and FK506 binding protein 5 (*FKBP5*) in adulthood in the amygdala. The increase in *FKBP5* expression was associated with decreased DNA methylation in *Fkbp5* intron *V*. These results indicate a sex-specific response to maternal programming by prenatal predator odor exposure and a potential epigenetic mechanism linking these responses with modifications of the stress axis in females. These results are in accordance with the mismatch hypothesis stating that an animal's response to cues within its life history reflects environmental conditions anticipated during important developmental periods and should be adaptive when these conditions are concurring.

1. Introduction

The mother is the primary vector of transmission by which environmental information in utero and during early postnatal period is transmitted to her offspring, and has been suggested to prepare the offspring for its future environment (Meaney, 2001; Welberg and Seckl, 2001). Both maternal physiology and maternal behavior can lead to long-term 'programming' of several aspects of offspring phenotype that persist throughout adulthood. The hypothalamic-pituitary-adrenal (HPA) axis, a prime regulator of the endocrine response to stress, is known to be sensitive to prenatal stress and potentially associated reductions in maternal behavior that program a heightened sensitivity to stress (Darnaudéry and Maccari, 2008; Merlot et al., 2008). There is increasing evidence that early life stress has lasting effects on neurobiological outcomes in wild animals, leading to calls for rapprochement between field and laboratory scientists towards identifying mechanisms involved in these processes (Clinchy et al., 2011; Love et al., 2013). Similarly, the mismatch hypothesis, originating in the ethology and evolutionary biology literature, has been proposed to understand ultimate causes of adaptations to the prenatal and early postnatal environment that lead to long-term pathological outcomes in mismatched prenatal and postnatal environments (Gluckman et al., 2011).

In prey animals such as rodents, predator odor is a stressor that is ecologically relevant, unconditioned and psychogenic. Predator odor exposure has been associated with a wide range of impacts in prey species, including a long-term increase in anxiety behaviors in natural settings, changes in activity and exploration, increased stress-hormone levels, heart rate and blood pressure, decreased body weight and altered transcript abundance of stress-induced genes (Adamec et al., 2004b; Campeau et al., 2008; Masini et al., 2005; Zoladz et al., 2008). These responses are, to some extent, species-specific. It has been proposed that an animal's response to cues within its life history reflect environmental conditions anticipated during important developmental periods and should be adaptive when these conditions are concurrent (Glover and Hill, 2012; Gluckman et al., 2011). Earlier studies in rodents have reported alterations in maternal behavior, anxiety-like behavior and object recognition memory in offspring exposed to predator odor during early postnatal life in a sex-specific manner (Ayers et al., 2016;

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Coutellier and Würbel, 2009; Mashoodh et al., 2009; McLeod et al., 2007). For example, in adulthood rat male offspring exposed to predator odor during early postnatal life show increased anxiety-like behaviors in the Open Field task while female offspring showed the opposite in the same task (Mashoodh et al., 2009). However, in these studies, offspring were directly exposed to predator odor during early postnatal life.

Few studies have investigated the impact of maternal exposure to predator or predatory cues during gestation on the offspring phenotype. In mice, exposure to predator rat urine during the first week of pregnancy lead to a decrease in the litter size and survival of the offspring (de Catanzaro, 1988). In rats, exposure to a live predator prenatally has been linked with a predisposition to seizures associated with alterations in hippocampal plasticity (Ahmadzadeh et al., 2011; Korgan et al., 2014; Saboory et al., 2011). We previously reported that prenatal predator odor (PO) exposure enhances PO-induced defensive and stress-related responses in adult mice (St-Cyr and McGowan, 2015).

The present study was designed to (1) study the impact of maternal PO exposure in offspring on anxiety-like behavior and locomotor responses associated with unconditioned novelty-induce anxiety in commonly used laboratory tests, (2) examine whether maternal PO exposure leads to increased defensive responses and predator odor-induced endocrine stress responses in offspring, (3) examine stress-related gene expression in limbic regions and associated epigenetic modifications in adult PO offspring and (4) highlight sex differences in the PO offspring adult phenotype. We used Long-Evans rats, an outbred rat strain in which the effects of chronic variable stress in the prenatal period and maternal behavior on offspring stress-related phenotypes have been extensively investigated (Erickson et al., 2014; Harmon et al., 2009; McGowan et al., 2011; Weaver et al., 2004).

We explored whether maternal PO exposure and potential consequent alterations in maternal behavior lead to long-term programming of stress-related behavior in the offspring. Pregnant rats were exposed to predator odor during the second half of pregnancy, a period of intensive foetal HPA axis development (Charil et al., 2010). We hypothesized that anxiety-like behavior, locomotor activity, predator odor-related defensive responses would be increased in PO animals as a result of enhanced HPA stress reactivity. Additionally, we assessed the activity of stress-related genes in the hippocampus and amygdala, limbic structures that have an inhibitory and activational effect, respectively, on HPA axis reactivity (Steckler, 2005). Finally, we sought to determine the extent to which sex differences in the response to predator odor reflect a general alteration in stress-related behavioral responses.

This study focused on the activity of stress-related genes in the hippocampus and amygdala, limbic structures that have an inhibitory and activational effect, respectively, on the HPA axis reactivity (Steckler, 2005). *Fkbp5* is a co-chaperone of multiple steroid receptors, including the glucocorticoid receptor (*Nr3c1*) and mineralocorticoid receptor (*Nr3c2*), and is essential for mediating the effect of those steroid hormones on gene expression via transactivation. In the nucleus, *Nr3c1* acts as a transcription factor and binds to glucocorticoid receptor elements (GRE) to alter transcription, including on the *Fkbp5* gene itself to potentiate its transcription, forming an ultra-short regulatory loop. *Fkbp5* expression is thus implicated in glucocorticoid sensitivity and stress recovery (Jääskeläinen et al., 2011). *Fkbp5* is ubiquitously expressed in tissues ranging from brain to liver and heart (Baughman et al., 1997). In the mouse brain, glucocorticoids lead to an up-regulation of *Fkbp5* expression in the paraventricular nucleus, central amygdala and hippocampus. For example, restraint stress increases the expression of *Fkbp5* (Jääskeläinen et al., 2011). Furthermore, *Fkbp5* polymorphisms have been implicated in mood, affective and anxiety disorders as well as post-traumatic stress disorder development (Binder, 2009; Binder et al., 2008). Interestingly, intronic elements of the *Fkbp5* gene exhibit demethylation after chronic glucocorticoid exposure through a conserved main glucocorticoid response element (GRE) enhancer and this difference persists over time (Hubler and Scammell, 2004) making *Fkbp5* an interesting target for long-term programming effects of glucocorticoid exposure during HPA axis development.

2. Material and methods

2.1. Animal housing and breeding

Adult Long-Evans rat females were bought from Charles River Canada (St. Constant, Canada). Females were housed in same-sexed groups (2 per cage) and maintained on a 12:12 h light-dark cycle (lights on at 7:00 am) with ad libitum access to food and water. Experimental protocols were approved by the Local Animal Care Committee at the University of Toronto, Scarborough, and were in accordance with the guidelines of the Canadian Council on Animal Care.

For breeding, two females were housed with a male between 9:00 AM and 5:00 PM. Females were then checked for sperm plugs indicating gestational day 0 (GD0). Females were weighed every other day throughout the pregnancy a brief manipulation equivalent to the weekly cage change. Pregnant females were singly housed. Twenty-one of the 23 females that were bred gave birth, resulting in 11 litters from predator odor-exposed dams and 9 control litters. One litter was excluded because of its small size (5 pups only).

2.2. Predator odor exposure in pregnant dams

Females were habituated to the exposure room, which included a fume hood for the predator odor exposure, for 5 consecutive days prior to breeding. From GD12 to 20, females were introduced for an hour to a small cage (19 × 39 cm) and presented with liquid odors on cotton balls sealed in a petri dish pierced with holes to avoid direct contact with the odorants. This apparatus made the odor presented inescapable and uncontrollable. Control females were exposed to distilled water at the same time each day while PO females were exposed three times each to 3 mL of bobcat urine, 5 mL of coyote urine (Pmart, Sandy Point, ME, USA) or 100 μL of a 1:5000 solution of 2,3,5-Trimethyl-3-thiazoline (TMT, a component of fox faeces; Contech Inc., Victoria, Canada, product number #300000368) dissolved in mineral oil in a randomized manner at a random time once a day. Bobcat and coyote urine elicit avoidance and defensive behaviors and elevated corticosterone (CORT) level (S.S. preliminary testing; St-Cyr and McGowan, 2015) while TMT is widely used in predator exposure studies (Mueller and Bale, 2006; Takahashi et al., 2008a,b; Wallace and Rosen, 2000). Preliminary analysis of serum CORT in non-pregnant females ($n = 4$ per group) showed a lack of habituation to predator odor exposure over 8 days as an interaction between the time and repeated predator exposure effect [$F_{(1,6)} = 12.331$, $P = 0.01$; $p\eta^2 = 0.67$] and an increased stress response to predator odor on the final day of exposure compared to distilled water control [$t_{(1,6)} = -5.488$, $P = 0.002$, $d = -3.9$; Fig. 1]. These results support the use of successive unpredictable exposures to predator odor to induce elevated stress responses.

2.3. Maternal behaviors in dams and offspring morphological measures

At postnatal day 0 (PN0), litters were culled to 8 pups (4 males and 4 females). Between PN1-6, maternal behaviors were video recorded for 1 h, 6 times a day throughout the rat's subjective light phase (7:00 AM, 11:00 AM, 3:00 PM) and dark phase (7:00 PM, 11:00 PM, 3:00 AM) (Champagne et al., 2007). Focal maternal behaviors including licking, nursing (high-crouch, low-crouch, supine), hovering, nest-building, as well as dam self-directed behaviors, including time off the nest, self-grooming, eating and drinking, were coded using Observer XT 8.5 every 3 min for a given observation (120 observations/day/animal). The percentage of maternal behavior was calculated by dividing the frequency observed by the number of observations made per day multiplied by 100.