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Rodent sex differences in disgust behaviors (anticipatory nausea) conditioned to a context associated with the effects of the toxin LiCl: Inhibition of conditioning following immune stimulation with lipopolysaccharide

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ABSTRACT

This study examined sex differences in the establishment of lithium chloride (LiCl)-induced conditioned disgust behavior (anticipatory nausea) to a distinct context in adult male and female rats. Also examined were potential sex differences in response to treatment with the bacterial endotoxin, lipopolysaccharide (LPS), and its effect on learning and memory. Twenty-nine male and 31 female naïve Long-Evans rats were injected (intraperitoneally; i.p.) with either 200 μg/kg LPS or 0.9% (NaCl), 90 min prior to i.p. injections of either 128 mg/kg LiCl or 0.9% NaCl, and immediately placed into a distinctive context for 30 min (repeated over 4 conditioning days, spaced 72 h apart). 72 h following the final conditioning day, each subject was re-exposed to the context on a drug-free test day where orofacial and somatic behaviors were recorded. Results showed that LiCl-treated females conditioned stronger disgust reactions, relative to LiCl-treated males, as evidenced by significantly higher frequencies of conditioned "gaping" behavior ($p < 0.02$) and forelimb flailing ($p < 0.01$) in females. Pre-treatment with LPS during conditioning led to strong inhibition of conditioned disgust behavior, to levels that did not significantly differ from controls. Although there was no apparent sex difference in the degree of inhibition produced by LPS in this context-based rodent disgust model, males did exhibit significantly greater 24 h body weight losses following LPS injections on the first two conditioning days, relative to females. The results of the current study provide strong evidence for a sex difference in the establishment of anticipatory nausea, as well as, evidence for a sex difference in the acute-phase response to endotoxin treatment.

1. Introduction

Chemotherapy treatment plays a major role in cancer survival rates; however, its cytotoxicity produces undesirable side effects, including severe acute and delayed nausea. This aversive nausea experience serves as an unconditioned stimulus in the establishment of a phenomenon termed Anticipatory Nausea and/or Vomiting (AN/V). Anticipatory nausea presents as a classically conditioned response, produced by the pairing of the hospital treatment room or context with the nausea produced by the chemotherapy, resulting in the establishment of a robust conditioned nausea/vomiting response upon re-entry into the hospital context on subsequent visits, prior to actual drug treatment (Hickok et al., 2003). It is estimated that 30% of patients suffer from conditioned anticipatory nausea, and report it as the most aversive component of chemotherapy treatment (Rodriguez, 2013). Due to the severity of this conditioned response, patient non-compliance increases as many choose to forego further treatment that could be life-saving (Molassiotis, 2005; Morrow et al., 1998).

Anticipatory nausea conditioning can be modeled in rodents by means of "conditioned disgust" learning and memory paradigms. Although rats are incapable of vomiting (Borison, 1989; Hatcher, 1924; Horn et al., 2013), they do produce a gaping response which is similar to the orofacial topography of the retch response exhibited by the shrew, *Suncus murinus*, just prior to an emetic response (see Parker et al., 2008; Parker and Limebeer, 2006), and involves the same musculature (Travers and Norgren, 1986). Conditioned disgust was initially demonstrated in rats by pairing a novel taste with the effects of a nausea-inducing emetic drug, such as lithium chloride (LiCl). Garcia et al. (1974) argued that the rat disgust behaviors of gaping and chin rubbing, observed in response to the conditioned taste cue following such conditioning, were established by an association between the taste and activation of emetic neural circuitry, such as the brainstem emetic

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chemoreceptor trigger zone (area postrema; Borison, 1989). By quantifying the rat orofacial and somatic behaviors when challenged with an intraoral infusion of the conditioned taste in a drug free test, Grill and Norgren (1978a) demonstrated that the disgust behaviors of gaping, chin rubbing and paw treading, were a strong index of conditioned taste aversions. Other studies have found that only emetic drugs condition gaping responses (Parker and Limebeer, 2006), and that the taste conditioned disgust response of gaping can be reduced or eliminated by lesioning the area postrema in rats (Eckel and Ossenkopp, 1996; Ossenkopp and Eckel, 1994, 1995), or, by treating the rats with antiemetic drugs, such as ondansetron and 8-OH-DPAT (Limebeer and Parker, 2000; Parker et al., 2008; Tuerke et al., 2012).

Exposure to a context previously associated with feelings of nausea elicits not only a robust conditioned context place avoidance (e.g., Tenk et al., 2005), but also a conditioned disgust response (gaping) and this paradigm has been offered as an animal model of anticipatory nausea (see Limebeer et al., 2006; Parker et al., 2008). Dose related increases in rat gaping behavior have been observed in a drug free test trial following multiple pairings of a novel context with the effects of LiCl (Ossenkopp et al., 2011). Thus, rats can learn and remember associations between distinctive environments and experienced nausea, and subsequently retrieve these associations to show aversion-related behaviors, such as gaping, upon re-exposure to this environment (Limebeer et al., 2008). This model can serve as a valuable preclinical tool for examining anticipatory nausea in chemotherapy patients (Limebeer et al., 2008; Molassiotis, 2005; Parker et al., 2008). As this population comprises one third of all patients undergoing chemotherapy treatment, individual differences, such as sex differences, in AN/V should be examined in order to determine the possible reasons why some patients establish strong anticipatory nausea, while others do not.

In addition to examining sex differences in the establishment of conditioned disgust, it is also important to evaluate sex differences in the efficacy of drug treatments that have been shown to disrupt learning and memory in these paradigms. Lipopolysaccharide (LPS) treatment is an experimental procedure that has been shown to reduce or abolish the conditioning of disgust responses to novel taste or context cues in rats (Chan et al., 2009, 2013; Cloutier et al., 2012a,b; Cross-Mellor et al., 2009). LPS is the active component of the cell wall of Gram negative bacteria, and when injected into an animal results in the production of pro-inflammatory cytokines (Dinarello, 1984), chiefly interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). LPS treatment also initiates a specific set of pathophysiological and behavioral changes collectively known as "sickness behaviors". These "sickness behaviors" include induction of fever (Hart, 1988; O'Reilly et al., 1988; Roth et al., 1997), anorexia and adipsia (Cross-Mellor et al., 2009; Gayle et al., 1998; Langhans, 2000; Langhans et al., 1990), reductions in locomotor activity (Hart, 1988; Engeland et al., 2003a; Franklin et al., 2003; Yirmiya et al., 1994), hypersomnia and reduction in grooming (Hart, 1988), helping the organism counter the bacterial infection.

LPS or IL-1β treatment have been found to have deleterious effects in some learning and memory paradigms. Studies have found effects on the consolidation phase in contextual fear conditioning, context discrimination, and two-way active avoidance learning (e.g., Pugh et al., 1998; Kranjac et al., 2012; Oitzel et al., 1993; Pugh et al., 2001; Sparkman et al., 2005a; Yirmiya et al., 2002). However, the nature of the sickness behaviors produced by LPS treatment can make it difficult to separate cognitive effects from changes in locomotor output. For example, some studies have shown learning and memory impairments in spatial reference learning in the Morris water maze (Arai et al., 2001; Shaw et al., 2001; Sparkman et al., 2005b) and in the radial arm maze (Song et al., 2004). However, other studies have considered the influence of LPS-induced reductions in locomotor output on spatial performance and have concluded that latencies to reach the hidden platform in LPS-treated rats are due to decreased swimming speeds, as opposed to increased distances travelled to reach the platform (Cunningham and Sanderson, 2008; Sparkman et al., 2005b). The conditioned disgust (gaping) paradigm is not influenced by locomotor reductions (Grill and Norgren, 1978b).

The conditioned disgust rodent model of anticipatory nausea has to date only used male subjects. In the human population, anticipatory nausea occurs more frequently in female patients (Fetting et al., 1983; Hilarius et al., 2012; LeBaron et al., 1988). Thus, it was of interest to examine the conditioning of nausea related disgust responding in female rats. Females also exhibit enhanced immune functioning, relative to males (e.g., Bilbo and Nelson, 2001; Engeland et al., 2003a,b; Gaillard and Spinedi, 1998; Lahita, 2000), and such effects on learning and memory require further examination. The current study investigated the effects of treatment with the bacterial endotoxin LPS on the establishment of LiCl conditioned disgust reactions in both male and female rats. As women exhibit anticipatory nausea more frequently than men, it was predicted that a sex difference in the frequency of conditioned disgust responses would be observed, evidenced by a higher frequency of conditioned disgust reactions in the female rats, relative to the males. Given this specific objective of the present study, a priori pairwise comparisons for sex differences in conditioned disgust behaviors were applied. Secondly, as females show enhanced immune functioning, relative to males, it was predicted that a sex difference in response to LPS treatment would be observed in terms of acute phase sickness behaviors (less weight loss) during conditioning and in its effects on learning (less reduction in conditioned disgust responses).

2. Materials and methods

2.1. Subjects

Subjects were 29 male and 31 female adult naive Long-Evans rats (Charles River, Quebec, Canada) weighing between 175 and 250 g at the start of the experiment. The rats were pair-housed in standard polypropylene cages (45 × 22 × 20 cm) in a colony room with a temperature of 21 ± 1 °C and maintained on a 12-h light:12-h dark cycle with the lights on from 07:00 to 19:00 h. All rats had free access to food (ProLab RMH 3000 rat chow) and tap water throughout the experiment. The experimental methodology was carried out according to the Canadian Council on Animal Care guidelines and was approved by the University of Western Ontario Animal Care Sub-Committee.

2.2. Apparatus

The apparatus (used on all conditioning days and the test day) consisted of a white Plexiglas box (29 cm × 25 cm × 29 cm) set atop a clear glass plate. A mirror was mounted at a 45° angle beneath the glass plate in order to view the rat's ventral surface. Two 40 W red lights were placed below the glass plate (see Fig. 1B). Lighting cues were kept consistent with previous studies employing this rodent model of anticipatory nausea (e.g., Chan et al., 2009). Behavioral responses exhibited on the Drug-Free Test Day were videotaped with a video camera (Sony DCR-DVD201; London, Ontario) positioned approximately 1 m from the mirror.

2.3. Experimental procedure

An illustration of the testing injection schedule is provided in Fig. 1A–B. All conditioning and testing was performed during the light phase of the LD cycle. The experiment consisted of two phases, including a Conditioning Phase (4 days, spaced 72 h apart), and one Drug-free Test Day, 72 h following the final conditioning day. There were four experimental groups for each sex resulting in 8 groups ($n = 7$–8/group) in total.

2.3.1. Conditioning phase drug treatment

On each day of the Conditioning Phase (4 days, 72 h apart), animals were pre-treated with an intraperitoneal injection of either 200 μg/kg