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Pathogen threat and unfamiliar males rapidly bias the social responses of female mice

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There is mounting evidence that pathogen threat affects social preferences and responses. In humans, the presence of strangers also elicits heightened sensitivity to pathogen threat, promoting 'in-group' bias and 'out-group' avoidance. Whether or not a similar effect of social context on responses to pathogen threat occurs in nonhuman animals is unclear. Here we show that the responses of female laboratory mice, *Mus musculus*, to males are also rapidly affected by the presence of unfamiliar or infected males. In female mice, where odour cues drive appetitive and aversive social responses, brief (1 min) exposure to the urinary odours of an unfamiliar male led to females subsequently discriminating more strongly against the odours of males subclinically infected with the murine nematode parasite, *Heligmosomoides polygyrus*. Likewise, brief exposure to the odours of infected males attenuated the responses of females to the odours of the normally preferred unfamiliar males and enhanced their preferences for familiar males. These findings are consistent with the concept of 'assortative' sociality, whereby the presence of pathogen threat and unfamiliar individuals biases female preferences for uninfected and familiar individuals ('in-group' preference and, in human terms, 'ethnocentrism') and leads to the avoidance of unfamiliar individuals ('out-group' avoidance and 'xenophobia'). Hence, as in humans, social information associated with infection can rapidly bias the social preferences of female mice. These rapid shifts in social preferences can have implications for our understanding of the evolution of social interactions and group composition.

There is accumulating evidence that actual and potential pathogen threat affect social and sexual responses. Across species, parasites and infection, in general, have been shown to influence social preferences and responses (e.g. Able, 1996; Beltran-Bech & Richard, 2014; Hamilton & Zuk, 1982; Kavaliers, Choleris, & Paff, 2005; Martinez-Padilla, Veragra, Mougeot, & Redpath, 2012; Milinski & Bakker, 1990). For example, in rodents, where odour cues are of major importance in determining social responses (Arakawa, Cruz, & Deak, 2011; Hurst, 2009; Hurst & Beynon, 2004), female mice can distinguish between the odours of infected and uninfected males, displaying aversive responses to and avoidance of the odours of parasitized males (e.g. Ehman & Scott, 2001, 2002; Kavaliers et al., 2005; Kavaliers & Colwell, 1995; Penn, Schneider, White, Slev, & Potts, 1998; Zala, Potts, & Penn, 2004).

There is also a growing awareness of the need to consider recent social history, individual experience and social factors when examining the impact of infection on social preferences and mate choice (e.g. Beltran-Bech & Richard, 2014; Brooks, 2002; Cotton, Small, & Pomiankowski, 2006; Jennions & Petrie, 1997; Jordan & Brooks, 2011; Kavaliers et al., 2005; Rodriguez, Rebar, & Fowler-Finn, 2013). Rapid social effects on behaviour are now being increasingly recognized (Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009). Results of studies with mice have shown that brief exposure to the odours of infected males immediately and transiently attenuates females' choosiness in that they cease to avoid the odours of infected males (Kavaliers, Colwell, Braun, & Choleris, 2003). In humans, pathogen threat directly affects women's perceptions of male attractiveness by increasing negative attitudes towards and decreasing interest in and positive responses to

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unfamiliar and/or lower-quality males (Jones et al., 2013; Little, DeBruine, & Jones, 2011; Murray, Jones, & Schaller, 2013; Neuberg, Kenrick, & Schaller, 2011). This has promoted the idea of 'assortative sociality' in humans, whereby perceived pathogen infection threat favours social interactions between familiar individuals ('in-group' bias) with an increased sensitivity to and avoidance of unfamiliar individuals ('out-group' avoidance) (Faulkner, Schaller, Park, & Duncan, 2004; Fincher, Thornhill, Murray, & Schaller, 2011; Navarette & Fessler, 2006; Oaten, Stevenson, & Case, 2011).

The reverse relationship is also evident in humans, with the presence of an unfamiliar individual leading to a heightened sensitivity to pathogen threat and enhanced disgust and aversive responses (Jones et al., 2013; Little et al., 2011; Murray et al., 2013). Thus, in humans, there is a complex interplay between pathogen threat and social context, whereby the presence of strangers affects responses to pathogen threat and the presence of pathogen threat reduces positive responses to unfamiliar individuals. Hence, the immediate social context appears to 'fine-tune' the sensitivity to pathogen threat. However, whether or not these effects are specific to humans or generalize to other species is currently unknown.

Here, we investigated the interplay between pathogen threat, familiarity and social preferences in female laboratory mice, *Mus musculus*. Using an odour preference test, the results of which are considered to be consistent with social and sexual preference (Ehman & Scott, 2002; Krackow & Matuschak, 1991), we examined the effects of (1) brief exposure to the odour of either an unfamiliar or a familiar male on the responses of female mice to the odour of a male subclinically infected with the nematode parasite, *Heligmosomoides polygyrus*, and (2) brief exposure to pathogen threat (urinary odour of an infected male) on the subsequent responses of female mice to the odours of familiar and unfamiliar uninfected males.

METHODS

Animals

Outbred male and female mice (CD-1, Charles River, Canada) were individually housed in clear Plexiglas cages (25 × 5 × 20 cm) under a 12:12 h dark light:dark cycle (lights on 0800–2000 hours) with wood shavings bedding and food (Pro-Lab Chow, St Louis, MO, U.S.A.) and tap water available ad libitum. An outbred strain of mice was used as inbred strains can display poor discrimination between same-strain individuals (Nevinson, Armstrong, Beynon, Humphries, & Hurst, 2003). Parasitized and nonparasitized mice were kept separate throughout. To avoid accidental infection, immediately after the end of the experiment all mice were humanely euthanized. All procedures were conducted in accordance with the Institutional Animal Care Committees (University of Western Ontario protocol number 2008-058-05; Agriculture and Agri-Food (Lethbridge) protocol no. LRC 172) and the guidelines of the Canadian Council for Animal Care.

Parasite Infection of Males

Male mice were orally infected with approximately 400 infective (L3 stage) of *H. polygyrus* with their bedding collected and frozen 20 days after infection. This subclinical infection elicits no evident malaise or pathology, although it is considered sufficient to elicit immune alterations (Barnard, Behnke, Gage, Brown, & Smithurst, 1998; Kavaliers, Colwell, Braun, & Choleris, 2003). Resistant stages of *H. polygyrus* are shed in the faeces of infected hosts and, after a short development period, they are infective to other mice that acquire them during feeding, grooming and other social interactions (Hernandez & Sukhedo, 1995). Detailed descriptions of infection with *H. polygyrus*, collection of odours, along with the results of previous determinations of the responses of females to the odours of infected males and to the odours of uninfected restraint-stressed (control) males are provided elsewhere (Kavaliers et al., 2003). Briefly, urine was obtained by palpation from single males (Kavaliers et al., 2003) and frozen at −18 °C until use. At the end of the study, male mice were euthanized and infection was confirmed by necropsy.

Experimental Apparatus

Odour preferences of individual oestrous female mice ($N = 10$, per group) were determined in the light period in a clean cage (25 × 5 × 20 cm) into which a vented Plexiglas tube (10 cm in length, 3 cm in diameter, divided in the middle and sealed at each end with fine plastic mesh; Fig. 1) was placed. The mesh was sufficiently open to permit diffusion of the odours and to allow the female to have nasal contact with both volatile and nonvolatile odour components but prevented direct contact with and chewing of the odour sources. This eliminated the possibility of female infection while allowing full olfactory exposure.

General Experimental Procedures

Habituation to apparatus

To minimize novelty responses of females and habituate them to the apparatus, we exposed females to the empty tubes in clean cages for 30 min on 3 consecutive days before the test day.

Odour pre-exposures

Fifteen minutes prior to testing, we pre-exposed oestrous females to male odours. Different females ($N = 10$) were used for each test. During the odour pre-exposures, female mice were placed for 1 min in clean cages in a sealed Plexiglas portioned area (12.5 × 15 × 10 cm) of the cage that was provided with the vented Plexiglas tube in which an odour source was placed (Fig. 1). This small space ensured that the female was in close proximity and exposed to the odours.

As the odour source ($N = 10$, for each condition) for pre-exposure, we used 50 g of bedding from (1) an unfamiliar

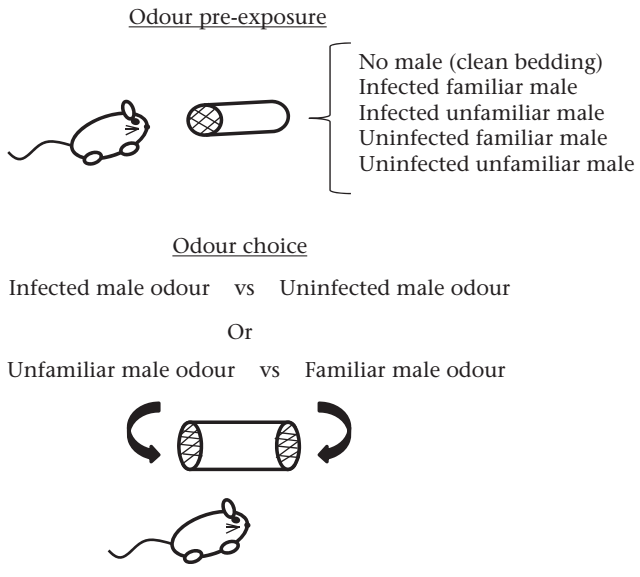

Figure 1. Schematic of the general experimental procedures used for odour pre-exposures and subsequent odour preference tests.