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An immune challenge reduces social grooming in vampire bats

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Social interactions affect the transmission of many pathogens, but infections often induce sickness behaviours that alter those interactions. Vampire bats are highly mobile and social, engaging in frequent allogrooming, which is likely to facilitate pathogen spread. Sickness behaviour is known to reduce social associations, but the effect on physical interactions between associated individuals, such as grooming, is less understood. Here, we tested the effects of induced sickness behaviour on allogrooming in vampire bats, while holding association between individuals in groups constant. To experimentally induce sickness behaviour, we used injections of lipopolysaccharide (LPS) and saline controls in 13 female common vampire bats, *Desmodus rotundus*, housed in stable groups of two to four adult bats. LPS injection induced an immune response that mimicked illness. Circulating leukocytes and neutrophil:lymphocyte ratios increased, while body mass and activity decreased. While LPS-injected bats did not receive less grooming from their group mates, they dramatically reduced the amount that they groomed their partners. This reduction in social interactions illustrates that sickness behaviour can potentially change transmission rates by altering directed behaviours, even under conditions of constant close proximity. The ability to manipulate social behaviours under controlled conditions should also prove useful for experiments attempting to test mechanisms underlying cooperation.

Animals often reduce their activity when sick (Hart, 1988; Kelley et al., 2003). Such 'sickness behaviours' probably aid in recovery from an infection (Hart, 1988; Kelley et al., 2003) and might also reduce transmission of pathogens to kin (Shakhar & Shakhar, 2015). Sickness behaviours can influence transmission rates by changing how individuals associate and interact. Sick animals might associate with more or fewer individuals (Lopes, Block, & König, 2016), where 'associations' are defined as individuals being at the same place at the same time (Franks, Ruxton, & James, 2010; Whitehead & Dufault, 1999). Such changes in associations could be caused by differences in how often sick individuals move towards conspecifics or merely how much they move around in general.

Even if sick individuals are near the same number of conspecifics, they might spend more or less time with each partner. They might also change how much they perform partner-directed behaviours such as mating, biting or grooming. By altering rates of interaction, it is possible that sickness behaviour can influence transmission rates despite not producing any detectable change in proximity-based associations. Although interaction rates are far more difficult to measure, interactions are likely to be better predictors of transmission rates than mere co-occurrences at the same site.

To disentangle these effects, one approach is to independently test the effect of sickness on both, associations (Lopes et al., 2016) and interactions (e.g. mating, Lopes & König, 2016). To test for effects of sickness on interactions between constantly associated common vampire bats, *Desmodus rotundus*, we took the first step of testing physiological and behavioural responses to an immune challenge under well-controlled conditions, where all individuals could be reliably observed and identified and were held in close proximity to each other. This scenario allowed us to measure changes in directed interactions while controlling for spatial proximity, before conducting tests of behavioural effects on freely interacting common vampire bats under less controlled conditions.

Vampire bats frequently groom each other by licking each other's fur, wings and face (Wilkinson, 1986). Vampire bats allogroom more than other bat species that have been observed, and females allogroom more than males (Carter & Leffer, 2015;

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Wilkinson, 1986). Allogrooming in vampire bats appears to help maintain long-term cooperative relationships that also involve regurgitations of blood to unfed bats (Carter & Wilkinson, 2013, 2015b; Wilkinson, 1984).

The high rates of allogrooming in vampire bats could be used by parasites and pathogens as a transmission pathway, especially because groomers often lick healed or open wounds (G. G. Carter, personal observation). Vampire bats are highly social and mobile vectors, and as obligate blood-feeders they frequently bite and lick the wounds of their hosts. They are the primary reservoir of rabies virus in Latin America (Johnson, Arechiga-Ceballos, & Aguilar-Setien, 2014; Streicker et al., 2012), but they can also be infected by other viruses or bacteria such as *Bartonella* (Becker et al., 2018; Wray et al., 2016), and *Leptospira* (Matthias et al., 2005). As mammalian parasites of multiple host species, they pose a unique risk for pathogen spillover (Johnson et al., 2014). The potential role of allogrooming for disease transmission in vampire bats is evident by culling practices that rely on a socially transmitted poison, sometimes called 'vampiricide'. After being applied to the fur of captured individuals, the poison is transmitted to others through social grooming, leading to the death of many group mates for each bat treated with the poison (Gomes, Uieda, & Latorre, 2006; Streicker et al., 2012).

To induce sickness behaviours without an infectious pathogen, we used lipopolysaccharide (LPS), a bacterial endotoxin that can simulate an infection (e.g. Schneeberger, Czirjak, & Voigt, 2013; Stockmaier, Dechmann, Page, & O'Mara, 2015). If LPS triggers an immune response, then LPS-injected vampire bats should show increases in white blood cell concentration (Schneeberger et al., 2013), changes in leukocyte composition (Rose, Banerjee, & Ramaiah, 2007), reduced mass (Schneeberger et al., 2013; Stockmaier et al., 2015) and lower activity (Hart, 1988; Kelley et al., 2003). We first verified these physiological and behavioural responses. We then tested whether sickness alters allogrooming rates within dyads. If healthy bats avoid sick individuals (Behringer, Butler, & Shields, 2006; Kiesecker, Skelly, Beard, & Preisser, 1999; Tobler & Schlupp, 2008; Zylberberg, Klasing, & Hahn, 2013), then sick bats should receive less allogrooming. Alternatively, if healthy bats direct allogrooming towards distressed individuals, then sick individuals should receive more allogrooming. If sickness behaviour serves to conserve energy (Hart, 1988; Kelley et al., 2003) or reduces transmission of pathogens to kin (Shakhar & Shakhar, 2015), then sick individuals should groom others less. Finally, we used a simple model to help illustrate when changes in rates of social interaction should most alter rates of disease transmission.

METHODS

Subjects

We captured 15 female common vampire bats exiting from a roost in Tolé, Panamá and another seven females flying together at a cattle pasture in Las Pavas, Panamá, and housed them together in captivity. We then divided these 22 bats into seven groups (four quartets and three pairs in 28 × 28 × 40 cm clear plastic observation cages). To control for past social experience, quartets (groups 1–4) included three females from the Tolé location and one female from the Las Pavas location and pairs (groups 5–7) included one female originally captured from each roost. The Las Pavas females from different roosts (one in each group) therefore had the exact same duration of familiarity with their group mates across groups. To feed the bats, we provided defibrinated bovine blood for 10–12 h every night. Each bat was identifiable by a unique combination of forearm bands and a subcutaneous passive integrated transponder (Trovan Ltd, U.S.A., www.trovan.com).

Experimental Treatments

Each focal bat ($N = 14$) was housed with one or three cage mates (22 bats total, see Appendix for details of group composition). The aim of testing pairs and quartets was to establish an immune challenge experiment in vampire bats in a highly controlled and easily observable environment, and to keep the spatial proximity between individual bats constant. To measure changes in dyadic allogrooming, we compared responses to LPS during a treatment period relative to pre-treatment and post-treatment periods over the course of a week. We also measured immediate changes in physiology and activity. For the pre-treatment period, we observed untreated bats for 2 nights. The treatment period started on night 3 when one randomly chosen bat in each cage was injected under the dorsal skin with LPS (L2630 Sigma-Aldrich, St Louis, MO, U.S.A.; 5 mg/kg lipopolysaccharide in phosphate-buffered saline). This dose was chosen because a similar dose caused physiological symptoms in another bat species without lingering effects (Stockmaier et al., 2015). The other bats in each cage received a control injection of phosphate-buffered saline (PBS). During the post-treatment period, bats were observed on nights 5–7 (for detailed experimental timeline see Appendix, Fig. A1). After a 1-night break, this week-long procedure was repeated with a different bat in each group now receiving the LPS treatment. Seven bats received LPS in the first week and six other bats received LPS in the second week, because one bat was removed from the experiment in the second week (for details, see Appendix, Table A1). In addition to these 13 treated subjects, six of the eight remaining cage mates received the control injection twice, one bat received one control injection in the first week and one bat did not receive any injections for health reasons (for details, see Appendix, Table A1). We compared responses within bats (physiological response and individual behaviour response) and within dyads (allogrooming response).

Physiological Responses

We measured body mass and sampled ~15 µl blood of each injected bat immediately before and 24 h after the LPS or PBS injection (see Appendix, Fig. A1). We sampled blood from the antebrachial vein using sterile needles and heparin-coated pipet tips. To determine the concentration of circulating leukocytes, we produced blood smears and stained them using a three-step differential haematology stain (Neat Stain, Astral Diagnostics, Paulsboro, NJ, U.S.A.). To measure immune response, we determined the ratio of neutrophils to lymphocytes by counting 50 specimens of either type under a light microscope at 400× magnification and dividing their respective counts. To measure concentration of circulating leukocytes, we haemolysed red blood cells and stained leukocytic nuclei by mixing whole blood with Turk's solution (crystal violet, 0.1% v/w in 1% filtered acetic acid) in a 1:10 ratio, then used a Neubauer haematocytometer (Bright-Line™, Sigma–Aldrich) to count leukocytes and determine their concentration in each sample. We first calculated the change in each parameter by subtracting the pre-injection value from the post-injection value. To test whether LPS affected the change in body mass, leukocyte concentration or neutrophil:lymphocyte ratio, we fitted null general linear mixed effect models that included the change as the response variable and injected bats nested in group as random effects, and final models that also included treatment (LPS, control) as a fixed effect. Subsequently, model fits were compared using maximum likelihood chi-square tests. Means and 95% confidence intervals were calculated using bootstrapping (described below).