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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).

Individual Behaviours

To record behavioural responses, we videorecorded each of the seven groups every night from 1800 to 0600 hours using a set of infrared surveillance cameras. We recorded bats at night because social interactions are most common during the night. To compare individual behaviours of bats injected with LPS or PBS, we used 1 h focal sampling to score behaviours of treated bats at 3 and 6 h post-injection (see Appendix, Fig. A1). For each hour of the video, a video-scorer that was blind to the treatment scored the focal subject at every 30 s instant as being in one of six mutually exclusive behavioural states: sleeping (appears asleep with eyes closed and wings folded), moving (changing location in the cage), self-grooming (scratching, chewing or licking its own body), allogrooming (grooming another bat's body), mouth licking (licking another bat's mouth) or offscreen (on or near the floor outside the camera frame), resulting in 120 behavioural samples per bat. Focal subjects were onscreen for an average of 98% of the sampled time (range 69–100%). We first calculated behavioural rates by dividing the number of samples of each behaviour by the number of samples the bat was onscreen. For each behavioural state, we then tested whether the mean difference in behaviour rate between LPS and the PBS control treatment differed from zero using a paired permutation test (5000 permutations). We also used permutation to test for an effect of group size (quartet versus pairs) on the difference between LPS and PBS. Means and 95% confidence intervals were calculated using bootstrapping (described below).

Dyadic Allogrooming

We tested for changes in allogrooming given and received across dyads of bats. To compare effects of LPS and PBS on the change in dyadic allogrooming interactions, treatment-blind observers scored the identity of the actor and receiver and the duration (s) of sampled allogrooming events for 2 h at times 3 and 6 h post-injection on injection nights and at equivalent time points on baseline nights. We defined events as allogrooming bouts longer than 5 s, and we recorded two separate events when two sequential grooming bouts were more than 5 s apart, following Carter and Leffer (2015). Sampling periods were 2 h except for nights 5 and 13, where 1 h of footage was lost due to power outage. To control for this, and to normalize the distribution of responses, we created a dyadic allogrooming index, defined as the natural log of (allogrooming seconds per hour +1). We then analysed how LPS affected this index using a permutation test. We first computed the mean change in allogrooming index from baseline to treatment for each dyad, and then computed the difference between the LPS versus PBS treatments. To test whether this difference was greater than expected by chance, we compared this mean difference to those from 5000 null data sets where we permuted the labels of PBS and LPS, within the same post-injection actor–receiver dyad. This permutation test therefore controlled other factors besides injection type.

Statistical Analysis, Data Visualization and Availability

Plots and analysis were generated in R version 3.3.2 (R Core Team, 2014) using the package 'lme4' for linear mixed effect models (Bates, Mächler, Bolker, & Walker, 2015), and the 'boot' package for calculating confidence intervals (Canty & Ripley, 2017). To calculate nonparametric 95% confidence intervals around means, we bootstrapped 10 000 times using the BCa method (Puth, Neuhäuser, & Ruxton, 2015). Both the data and the R scripts are publicly available on Figshare (Stockmaier, 2018).

Ethical Note

All experiments were approved by Panamanian authorities (MiAmbiente protocols SE/A-102-16 and SE/A-99-15) and the Animal Care and Use Committees of the Smithsonian Tropical Research Institute (protocol ID: 2016-0728-2019 and 2015-0915-2018) and University of Texas at Austin (protocol ID: AUP-2016-00124). After the experiments were concluded, bats were moved to a captive colony for another experiment investigating the formation of social bonds (IACUC protocol ID: 2015-0915-2018 to G.G.C.).

RESULTS

Compared to control treatments, treatment with LPS increased the change in circulating white blood cells from -2.66×10^5 cells/ml to 6.77×10^6 cells/ml (chi-square test: $\chi^2_1 = 26.88$, $P < 0.001$; Fig. 1a, Table A2) and the neutrophil:lymphocyte ratio from −0.14 to 4.63 (chi-square test: $\chi^2_1 = 25.11$, $P < 0.001$; Fig. 1b, Table A2). Compared to control treatments, LPS treatment also decreased the change in body mass from −0.56 g to −2.69 g (chi-square test: $\chi^2_1 = 22.40$, $P < 0.001$; Fig. 1c, Table A2).

LPS decreased the average percentage of time spent active (from 19% to 4%, $N = 13$, $P < 0.001$; Fig. 2a, Table A2), awake (from 30% to 15%, $N = 13$, $P = 0.001$; Fig. 2f, Table A2), self-grooming (from 12% to 0.8%, $N = 13$, $P < 0.001$; Fig. 2b, Table A2) and moving (from 4% to 2%, $N = 13$, $P = 0.080$; Fig. 2d, Table A2). Rates of mouth licking (a proxy for food begging) were low for both treatments, and we did not detect a significant decrease (from 0.5% to 0.1%, $N = 13$, $P = 0.188$; Fig. 2e, Table A2). We failed to detect a significant decrease after LPS injections in allogrooming rates of the 13 subjects at the whole group level (from 2.16% to 0.60%, $N = 13$, $P = 0.158$; Fig. 2c, Table A2), but see within-dyad effects below. We did not detect that group size influenced the effect of treatment (see Table A4).

In our analysis to test for LPS-induced changes in allogrooming given and received within dyads of bats and throughout baseline and injection nights, we recorded 558 individual dyadic allogrooming bouts across all groups, totalling 9.9 h of recorded allogrooming out of a total of 182 h of observation. This overall proportion of time spent allogrooming (5.4%) is consistent with observations of female vampire bats that were either in the wild (5%, Wilkinson, 1986) or in much larger groups in a flight cage (5.4%, Carter & Leffer, 2015). After controlling for factors besides treatment type, we found that an LPS injection decreased a bat's allogrooming of a given partner by 97% (permutation test: $P = 0.019$; Fig. 3b, Table A3), but we did not detect an effect on allogrooming received (permutation test: $P = 0.269$; Fig. 3a, Table A3). Permutation results are shown visually in the Appendix, Fig. A3.

DISCUSSION

We used a standard immune challenge to test how sickness behaviour affects allogrooming interactions in vampire bats. LPS injections caused loss of body mass and increases in circulating white blood cells and neutrophil:lymphocyte ratios, similar to other bat species (Schneeberger et al., 2013; Stockmaier et al., 2015) and rats (Rose et al., 2007). As expected from sickness behaviour (Hart, 1988; Kelley et al., 2003), LPS-injected bats also showed decreased overall activity, increased sleep and drastic reductions in self-grooming.

Sickness behaviours can serve as a social cue and induce avoidance behaviours by others (Zylberberg et al., 2013). Avoidance of sick individuals has been found in several species living in

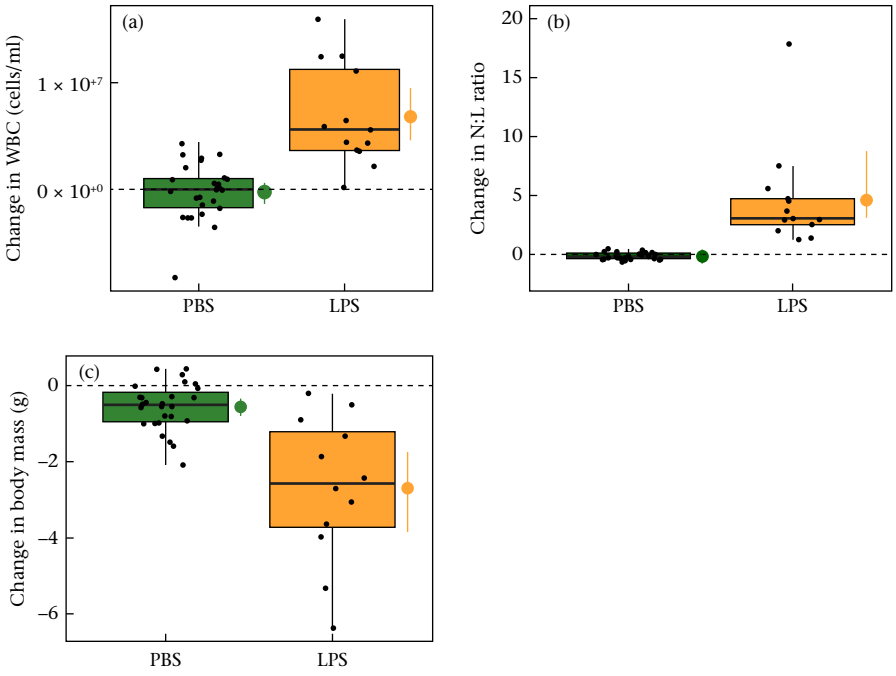

Figure 1. Effect of lipopolysaccharide (LPS) and control (phosphate-buffered saline, PBS) injections on physiology of captive female vampire bats. Box plots show the changes in (a) white blood cell (WBC) concentration, (b) neutrophil:lymphocyte ratio and (c) body mass 24 h post-injection. The dashed line represents no change. Adjacent points and errors bars show the means ± bootstrapped 95% confidence intervals (for details, see Appendix, Table A2).

conditions where it is possible to avoid diseased individuals (e.g. bullfrog tadpoles, *Rana catesbeiana*: Kiesecker et al., 1999; Caribbean spiny lobsters, *Panulirus argus*: Behringer et al., 2006; western mosquitofish, *Gambusia affinis*: Tobler & Schlupp, 2008; house finches, *Carpodacus mexicanus*: Zylberberg et al., 2013). However, the benefits of avoidance behaviours might decline for animals living in more stable social groups, because pathogen exposure may be unavoidable (Loehle, 1995). Vampire bats are known to live in unstable roosting groups with fission–fusion social dynamics, but females have stable social networks (Wilkinson, 1985). Individuals could potentially avoid sick conspecifics by grooming them less, but we did not find strong evidence for this. Studies on banded mongoose, *Mungos mungo* (Fairbanks, Hawley, & Alexander, 2015), rhesus monkeys, *Macaca mulatta* (Willette, Lubach, & Coe, 2007), and house mice, *Mus musculus domesticus* (Lopes et al., 2016), also found no evidence for avoidance of sick individuals. In these species and in vampire bats, the benefits of social interactions might outweigh the benefits of avoiding sick individuals.

LPS-injected vampire bats groomed themselves and their partners far less. Much variation in allogrooming existed across dyads. Although, we did not detect a decrease of allogrooming rates across individuals, we did detect a clear effect when controlling for the effect of the recipient's identity on the grooming interaction.

In house mice, LPS has been shown to reduce social exploration (e.g. Fishkin & Winslow, 1997), associations with others (Lopes et al., 2016) and interest in potential mates (Lopes & König, 2016). Unlike mere association, allogrooming in vampire bats likely represents a social investment of time and energy (Carter & Wilkinson, 2013, 2015b), and sickness-reduced allogrooming could therefore impose social costs. Consequently, sick bats might allocate more effort to continue allogrooming compared to self-grooming, consistent with evidence from other species where individuals suppress symptoms of behavioural sickness to participate in crucial social behaviours (reviewed in Lopes, 2014). For instance, LPS-injected zebra finches, *Taeniopygia guttata,* overcome the behavioural but not the physiological symptoms of sickness in the presence of conspecifics (Lopes, Adelman, Wingfield, & Bentley, 2012), and male song sparrows, *Melospiza melodia morphna,* suppress sickness behaviours during the breeding season (Owen-Ashley & Wingfield, 2006). We lacked the power to properly test this idea because LPS-influenced self-grooming and allogrooming rates were already low and bounded by zero (Fig. 3c), but our data are at least consistent with the possibility that LPS has stronger effects on self-grooming than on social grooming. For example, after control injections, all 13 bats groomed themselves more than others (Appendix, Fig. A2) as expected from past reports (Carter & Leffer, 2015; Wilkinson, 1986); however, when LPS injections reduced both types of grooming, three sick bats surprisingly spent more time grooming others than grooming themselves (Appendix, Fig. A2). This pattern warrants further investigation.

There are other reasons that pathogen-induced sickness behaviour might alter social behaviour differently than asocial behaviour. Most notably, sickness-induced behavioural outcomes may result from contrasting selection pressures on pathogens versus hosts. We used LPS, an inflammatory agent found in a variety of bacteria (Sutcliffe, 2010), to induce a general set of sickness symptoms (e.g. Fishkin & Winslow, 1997; Lopes et al., 2016; Willette et al., 2007), but many socially transmitted pathogens may not cause the decrease in social interactions we observed, or they may lead to different kinds of interactions. The most relevant case is rabies, which is linked to intraspecific aggression in vampire bats (Delpietro, Russo, Carter, Lord, & Delpietro, 2017). Allogrooming or food sharing might enable the rapid dissemination of rabies through vampire bat populations (Johnson et al., 2014), but the actual effects of rabies on these interactions remain unknown. We would expect natural selection to favour rabies virus variants that do not cause a decrease in social interactions.

Kin selection might also play a role in the evolution of sickness behaviour if it prevents disease transmission to kin (Shakhar & Shakhar, 2015). For example, when workers of the ant *Temnothorax unifasciatus* are dying from a fungal infection, they isolate themselves from their nestmates before death (Heinze & Walter,

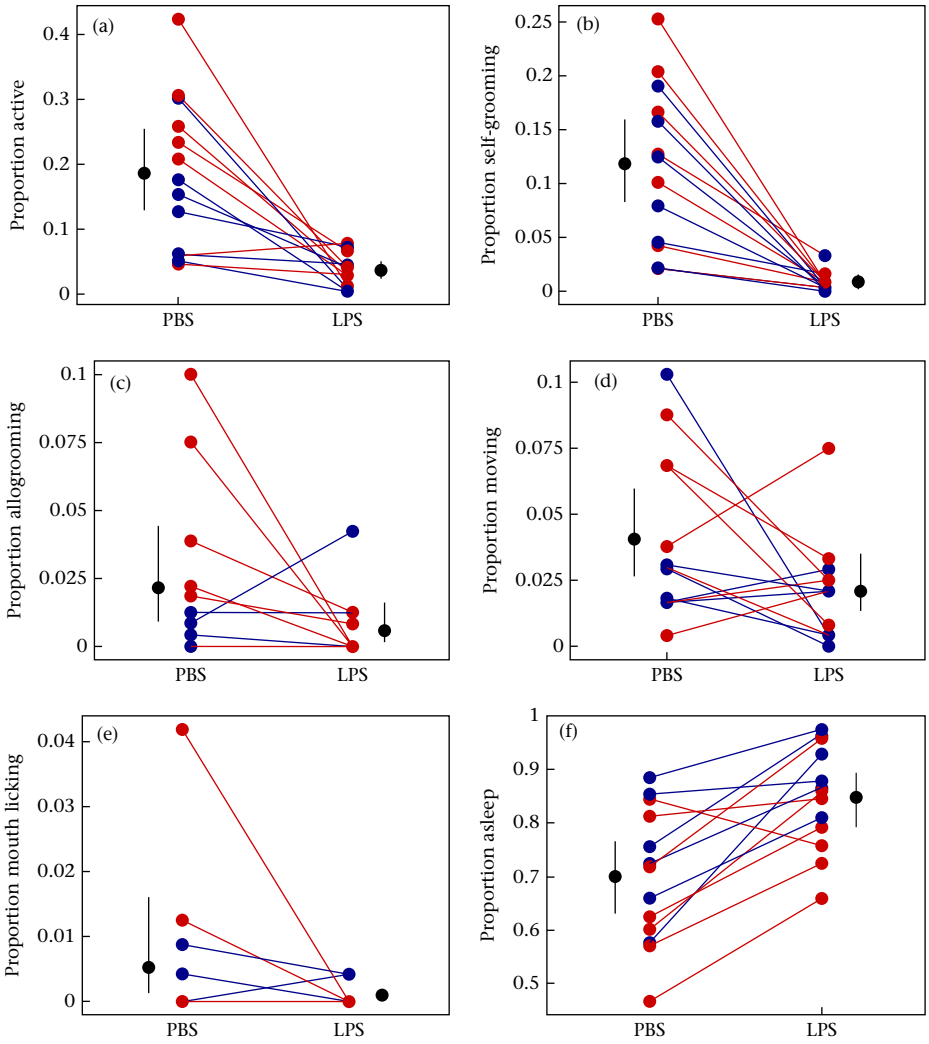

Figure 2. Effect of lipopolysaccharide (LPS) and control (phosphate-buffered saline, PBS) injections on individual behaviours of captive female vampire bats. Line plots show the proportions of time spent (a) active, (b) self-grooming, (c) allogrooming, (d) moving, (e) mouth licking, a potential indicator of food begging, and (f) sleeping following injection of LPS or PBS. Lines are coloured based on a focal individual's group size, with red for quartets and blue for pairs. Adjacent points and error bars are means ± bootstrapped 95% confidence intervals (for details, see Appendix, Tables A2, A4).

2010). We did not include kinship as a variable in our analysis because only two bat pairs were closely related. Future work, however, will test effects of both kinship and social history on LPS-induced sickness behaviour.

The impact of changes in any directed social interaction for disease transmission should depend on how easily the pathogen spreads by a social interaction. To illustrate this, consider a simple model for estimating the probability of transmission, $P(t)$, based on the duration of a directed social interaction.

$$P(t) = 1 - (1 - p)^t$$

where p is the infectivity of a pathogen (i.e. the probability of pathogen transmission from one individual to another per second of the respective social interaction). For various values of p, we can examine how much the observed LPS-induced shift of t in our study would affect $P(t)$, which increases with allogrooming rate. In our study, sick bats decreased their grooming of others from 24 s/h to 0.7 s/h, which would lead to lower disease transmission probabilities, especially when infectivity of the pathogen is low (Fig. 3d). If a pathogen is highly infectious, however, and spreads easily through mere association, there will be diminishing returns from decreases in social behaviour, because behavioural changes matter less for transmission. On the other hand, if a pathogen is less infectious, then such behavioural changes would matter relatively more for transmission. Hence, durations of social interactions are most important to consider in models of disease transmission when infectivity is low.

Our findings on LPS effects on directed interactions complement past studies investigating LPS effects on association by showing that interactions can be reduced even for animals held in constant association. Lopes et al. (2016) found that sickness-induced lethargy of LPS-injected house mice led to fewer associations and potentially lowered disease transmission. In our study, associations in groups were controlled by keeping bats in small cages, and interaction rates within dyads were reduced by LPS. The observed effects of sickness behaviour might differ between social networks based on association ('gambit of the group' associations; Franks et al., 2010; Whitehead & Dufault, 1999) versus social networks based on interactions like mating or allogrooming, because association samples do not always predict interaction rates in a straightforward manner (Castles et al., 2014). For example, a sick,

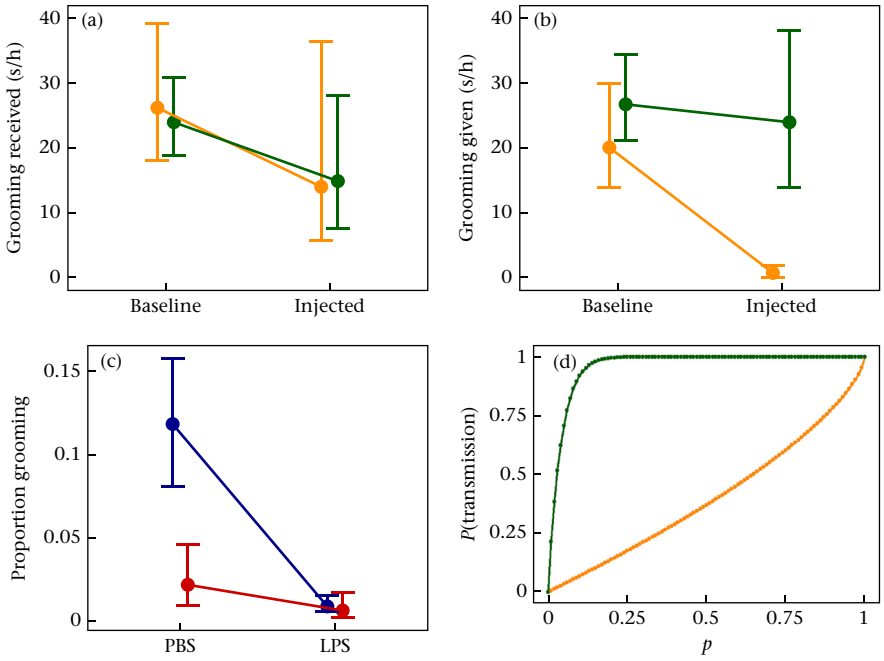

Figure 3. Effect of lipopolysaccharide (LPS) and control (phosphate-buffered saline, PBS) injections on allogrooming within directional dyads. Mean seconds per hour during which (a) allogrooming was received and (b) allogrooming was given by female vampire bats during baseline and following injection of LPS (orange) or PBS (green). (c) Mean proportion of time spent self-grooming (blue) and allogrooming (red) following LPS or PBS injection. Points and error bars represent the means ± bootstrapped 95% confidence intervals (for details, see Appendix, Table A3). (d) Estimated probability of transmission from the groomer to another individual following LPS (orange line) or PBS (green line) injection based on the rate of pathogen transmission per unit of social interaction.

lethargic individual that encounters fewer conspecifics will show a lower social connectedness, but the same individual might actually have more intense interactions with those individuals that it does encounter.

By manipulating social behaviours and experiences, LPS injections are a potential tool for testing the factors underlying cooperation. Past studies in vampire bats have used food deprivation to encourage food sharing (Carter & Wilkinson, 2015a, b; Wilkinson, 1984), simultaneous fasting to discourage food sharing (Carter & Wilkinson, 2015b), and oxytocin administration to increase sharing and grooming (Carter & Wilkinson, 2015a). LPS-induced lethargy can be used to suppress grooming or other directed social behaviours in targeted individuals, which would allow testing for long- and short-term responses of social partners. This method may not only be useful to manipulate social behaviour in vampire bats, but also in other study species.

To summarize, LPS-induced sickness significantly reduced allogrooming directed towards others within directional dyads of bats, even under conditions of constant proximity. Our findings show that sickness-induced changes in directed social behaviours within a group can have implications on the transmission of a pathogen and are likely to be most important for pathogens with low infectivity.

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Appendix

Table A1
Treatment details for bats in each group

	Week 1	Week 2
Group 1		
RC	LPS	PBS
SCS	PBS	LPS
LS[a]	PBS	–
EVE	PBS	PBS
Group 2		
SC	LPS	PBS
CCS	PBS	LPS
SSS	PBS	PBS
UNA	PBS	PBS
Group 3		
SD[b]	LPS	PBS
C	PBS	PBS
SCC[c]	PBS	–
DOS	PBS	PBS
Group 4		
S	LPS	PBS
CSC	PBS	PBS
SS[d]	–	–
TES	PBS	LPS
Group 5		
CC	LPS	PBS
CAT	PBS	LPS
Group 6		
DCD	LPS	PBS
IVY	PBS	LPS
Group 7		
DD	LPS	PBS
SIX	PBS	LPS

[a] LS received a control injection in the first week, but received no injection the second week to minimize stress, because she suffered some aggression from other bats. She is included in the analysis of the first week but not the second.
[b] SD aborted a very young fetus after LPS injection; her change in body mass with LPS injection was therefore not used in our analysis.
[c] SCC died the morning after the LPS injection in week 2, so we excluded her from the analysis for the second week.
[d] SS gave birth in the first night, so she was not injected in either week but was still allowed to interact with others in the group. Although she could function as a social partner, we did not include her in any tests on effects of injections. We did not include her pup as a social partner.

Table A2
Effects of LPS and PBS (control) on physiology and individual behaviour

	Treatment	Mean	95% CI	*N* (injections)
Change in white blood cell concentration (cells/ml) after injection	Control	-2.66×10^5	-1.42×10^6, 6.07×10^5	27
	LPS	6.77×10^6	4.58×10^6, 9.57×10^6	13
Change in neutrophil:lymphocyte ratio after injection	Control	−0.14	−0.25, −0.02	27
	LPS	4.63	3.17, 8.71	13
Change in body mass (g) after injection	Control	−0.56	−0.80, −0.34	27
	LPS	−2.69	−3.83, −1.77	12
% Time active after injection	Control	19	13, 25	13
	LPS	4	2, 5	13
% Time asleep after injection	Control	70	63, 77	13
	LPS	85	79, 90	13
% Time self-grooming after injection	Control	12	8.1, 16	13
	LPS	0.80	0.51, 1.5	13
% Time mouth licking after injection	Control	0.52	0.13, 1.7	13
	LPS	0.10	0, 0.19	13
% Time moving after injection	Control	4.10	2.67, 6.03	13
	LPS	2.10	1.32, 3.53	13
% Time allogrooming after injection	Control	2.16	0.92, 4.55	13
	LPS	0.60	0.16, 1.66	13

Means and 95% confidence intervals were calculated by bootstrapping.

Table A3
Effects of LPS and PBS (control) on dyadic allogrooming

	Treatment	Injection status	Mean grooming rate (s/h)	95% CI	*N* (observed dyads)
Grooming received	LPS	Injected	13.9	5.7, 36.4	27
		Baseline	26.2	18, 39.3	162
	Control	Injected	14.8	7.5, 28.2	66
		Baseline	24	18.8, 30.8	402
Grooming given	LPS	Injected	0.7	0, 1.8	27
		Baseline	20.1	13.9, 29.9	162
	Control	Injected	24	13.9, 38.2	66
		Baseline	26.7	21.1, 34.5	402

Means and 95% confidence intervals were calculated by bootstrapping.

Table A4
Paired permutation tests for individual behavioural rates

Behavioural state	*N* (bats)	Mean difference	Bootstrapped 95% CI of mean difference	Permutation *P* value
Activity	13	−0.15	−0.22, −0.09	<0.001
Sleeping	13	0.15	0.08, 0.20	0.001
Self-grooming	13	−0.11	−0.15, −0.07	<0.001
Mouth licking	13	−0.004	−0.02, 0	0.188
Moving	13	−0.02	0.04, −0.002	0.080
Allogrooming	13	−0.02	−0.04, 0	0.158

Paired permutation tests (5000 permutations) for differences (LPS − PBS) in behavioural rates of being active, asleep, self-grooming, mouth licking, moving and allogrooming. Paired permutation tests assessed whether differences were significantly different from zero. We calculated 95% confidence intervals for the mean differences by bootstrapping (5000 times, BCa method; Puth et al., 2015). If the BCa method for boostrapping was unstable we used the 'basic' method of the 'boot' package instead (Canty & Ripley, 2017). We also tested pairs and quartets separately; the conclusions were always the same for each subset and for all bats (Stockmaier, 2018).

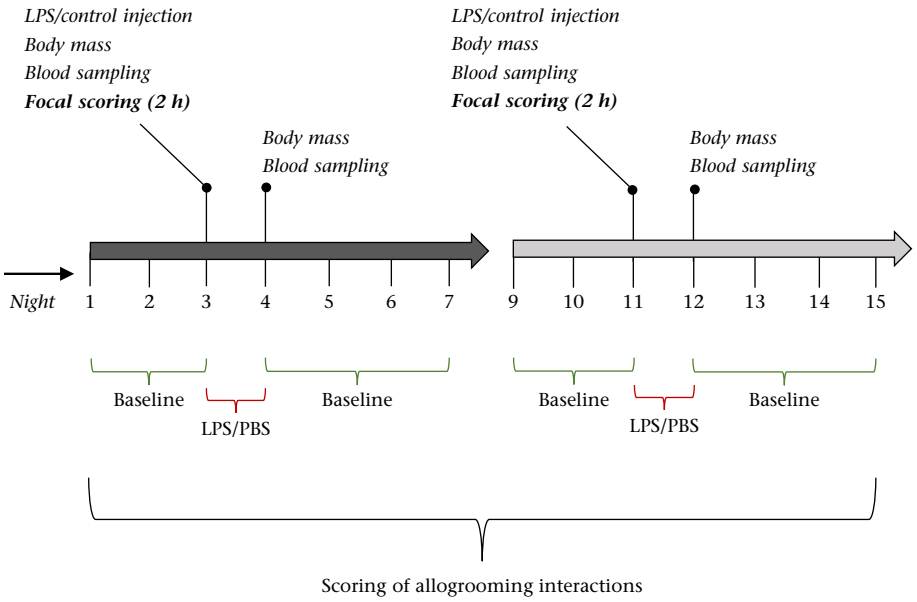

Figure A1. Experimental timeline. Social grooming interactions were scored 3 h and 6 h post-injection for 1 h, and during equivalent times for baseline nights. Due to technical problems, we lost 1 h of video recording on night 5 and night 13. In total, we recorded 26 h of each group (13 h in week 1, 13 h in week 2).

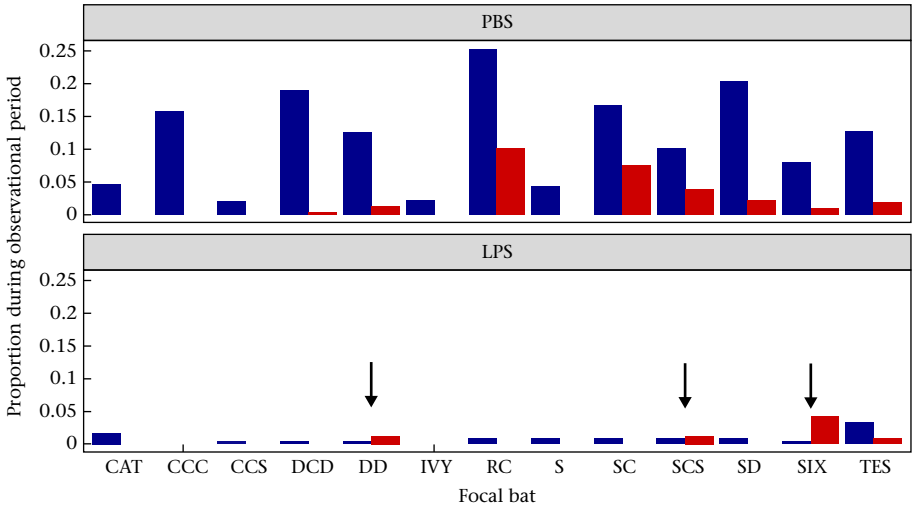

Figure A2. Self-grooming versus allogrooming in 13 female vampire bats after control (top panel) and LPS injections (bottom panel). Bars are rates of allogrooming (red) and self-grooming (blue). Arrows indicate only cases where rates of allogrooming exceeded rates of self-grooming.

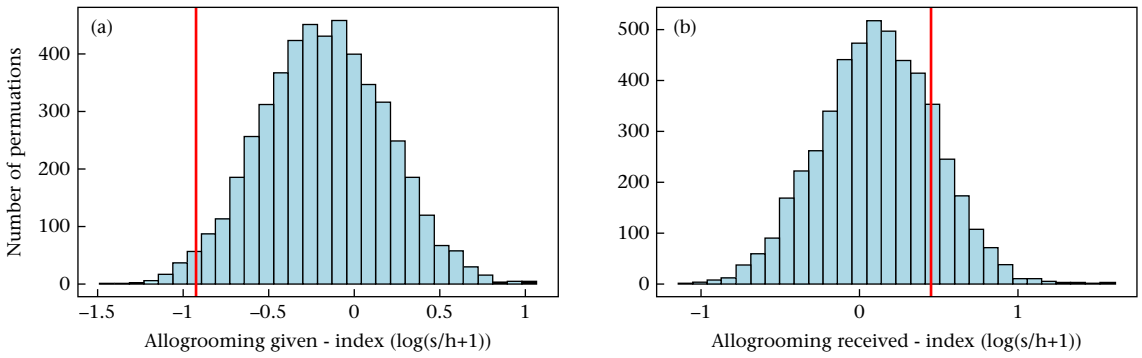

Figure A3. Permutation test results on effects of LPS on allogrooming. Expected mean changes in the rate of (a) allogrooming given and (b) allogrooming received (calculated by permuting the label of PBS or LPS within the factors of dyad and injection). The vertical red lines denote the observed values.