

## Original Article

# Sick bats stay home alone: fruit bats practice social distancing when faced with an immunological challenge

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Along with its many advantages, social roosting imposes a major risk of pathogen transmission. How social animals reduce this risk is poorly documented. We used lipopolysaccharide challenge to imitate bacterial infection in both a captive and a free-living colony of an extremely social, long-lived mammal—the Egyptian fruit bat. We monitored behavioral and physiological responses using an arsenal of methods, including onboard GPS to track foraging, acceleration sensors to monitor movement, infrared video to record social behavior, and blood samples to measure immune markers. Sick-like (immune-challenged) bats exhibited an increased immune response, as well as classic illness symptoms, including fever, weight loss, anorexia, and lethargy. Notably, the bats also exhibited behaviors that would reduce pathogen transfer. They perched alone and appeared to voluntarily isolate themselves from the group by leaving the social cluster, which is extremely atypical for this species. The sick-like individuals in the open colony ceased foraging outdoors for at least two nights, thus reducing transmission to neighboring colonies. Together, these sickness behaviors demonstrate a strong, integrative immune response that promotes recovery of infected individuals while reducing pathogen transmission inside and outside the roost, including spillover events to other species, such as humans.

**Keywords:** sickness behavior; immune response; social behavior; foraging; chiroptera

## Introduction

How social animals avoid transmission of pathogens among group members is a fundamental question in the study of animal sociality.<sup>1,2</sup> One main hypothesis argues that sickness behavior assists transmission prevention. Sickness behavior, as first described by Hart,<sup>3</sup> is a set of well-preserved<sup>4,5</sup> behavioral changes that ill individuals develop simultaneously with their illness, includ-

ing lethargy, depression, anxiety, malaise, loss of appetite, sleepiness, hyperalgesia, reduction in grooming and general movement, and a loss of interest in their surroundings. Although first believed to be merely a side effect of immunological processes, sickness behavior is now agreed to also have adaptive functions that support the physiological struggle of a sick individual against the infection, for example, by reducing energy expenditure.<sup>6</sup>

Sickness behavior has also been suggested as a mechanism to reduce transmission of pathogens to kin<sup>7</sup> and within the social group,<sup>8</sup> a feature that might be critical for animals living in dense social

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populations. A few documented examples include social isolation of sick individuals among eusocial insects, such as bees<sup>9,10</sup> and ants.<sup>11–14</sup> But so far, social isolation in vertebrates has only been shown to be a case of avoidance by healthy individuals; that is, healthy individuals avoid contact with sick ones. In multiple vertebrate species, healthy individuals can discriminate between sick and healthy conspecifics and spend more time in proximity to healthy than sick individuals.<sup>15–17</sup> Additionally, there is mounting evidence that sickness does not automatically induce classical sickness behaviors like lethargy but may be exhibited to varying degrees in species with different life-history strategies<sup>18</sup> or can be suppressed during social contexts.<sup>19</sup> Such variation demands the refinement of the classical sickness behavior hypothesis to account for interspecific and contextual variation. Importantly, sickness behavior has been rarely investigated in wild free animals<sup>20,21</sup> with only three studies of behavioral change in free wild mammals during sickness.<sup>22–24</sup>

Current knowledge of the metabolic and systemic changes during the first few days post injury or immunological challenge (acute phase response (APR)<sup>25</sup>) in bats suggests that different species might respond differently to similar threats. Immune responses have been measured in a few bat species using lipopolysaccharide (LPS), a bacterial endotoxin that induces an inflammatory response by stimulating the release of cytokines.<sup>26,27</sup> Fever has been documented in response to an LPS challenge in three out of four species examined (*Myotis vivesi*,<sup>28</sup> *Carollia perspicillata*,<sup>29</sup> and *Artibeus lituratus*,<sup>30</sup> but not in *Molossus molossus*<sup>31</sup>). Some species exhibit clear leukocytosis (*C. perspicillata*<sup>32</sup> and *Desmodus rotundus*<sup>33</sup>), while others shift the ratio of white blood cell (WBC) types without a clear overall increase (*Artibeus lituratus*<sup>30</sup>), and others do not increase WBC production at all (*M. molossus*<sup>31</sup>). Weight loss was observed in all instances body mass was measured, likely due to increased metabolic rate, as documented in the great fruit<sup>30</sup>- and fish-eating<sup>28</sup> bats, and decreased food consumption, as observed widely across animals<sup>5</sup> and recently documented in bats.<sup>34</sup> Molecular aspects of the immune response to bacteria are understudied in bats. One major acute-phase protein with bacteriostatic and immunomodulatory effects,<sup>35</sup> haptoglobin, increased following a fungal immune challenge.<sup>36</sup> Lysozyme, despite its key role in the immune response across species,<sup>37,38</sup> has only been recorded in bats as a component of the digestive system<sup>39</sup> and a measure of environmental disturbance.<sup>40</sup>

Many bats are extremely social, roosting in large colonies and clustering in tight groups. Still, sickness behavior and its role in preventing pathogen transmission is poorly studied in bats. In the only previously studied species, vampire bats (*D. rotundus*), LPS-injected individuals decreased overall activity levels, reduced grooming toward others, received less grooming from groupmates,<sup>33,41</sup> and had lower network centrality,<sup>23</sup> yet had no reduction in the number of food donors or the amount of food received following a night of food deprivation.<sup>41</sup> Such behavioral changes support the hypothesis that sickness behaviors alter interactions by which diseases are transmitted.<sup>7,33</sup>

We assess the APR and sickness behavior of the highly social Egyptian fruit bat (*Rousettus aegyptiacus*) in captive adults and free-ranging juveniles, using LPS to simulate an infection without an infectious pathogen. We expected a reduction in movement and food consumption and avoidance of sick-like individuals by healthy individuals. Unlike previous reports in other mammals, we found that sick-like individuals abandoned the social cluster and remained distant from conspecifics. This reaction was observed in both captive and free individuals. GPS tracking demonstrated that wild sick-like individuals failed to leave the roost to forage. We suggest the inflammatory response and sickness behaviors together serve to conserve resources while maximizing swift bacterial eradication and reducing transmission inside and outside the roost.

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## Methods

We established two consecutive colonies in the same closed room from a mixture of 19 recently caught male and 18 previously housed female Egyptian fruit bats (*R. aegyptiacus*) (see Supplementary Methods for full details on experimental animals and housing conditions, online only). Each colony contained five bats that were challenged via subcutaneous injection with LPS (Sigma-Aldrich, L2630) at a concentration of 4 mg/kg bw, (0.577 ± 0.144 mg) diluted in sterile phosphate-buffered saline (PBS; Sigma-Aldrich, P5493); five bats that

were injected with an equivalent amount of PBS as a control; and additional bats (13 in the first colony and 6 in the second colony) to provide a full social environment (see Supplementary Methods for full details on the immune challenge, online only). Following these trials, we challenged five young adult bats (approximately 7 months of age) in an open colony where bats have free access to nature at all times.<sup>42</sup> These individuals received a reduced dosage of 2 mg/kg bw ( $0.204 \pm 0.031$  mg) due to their age, and an injection of PBS at a separate time (three before LPS and two following LPS to counterbalance order), thus serving as their own controls (see Supplementary Methods for full details on the open colony, online only). All animals involved were assessed by an experienced veterinarian and deemed clinically healthy before beginning data collection.

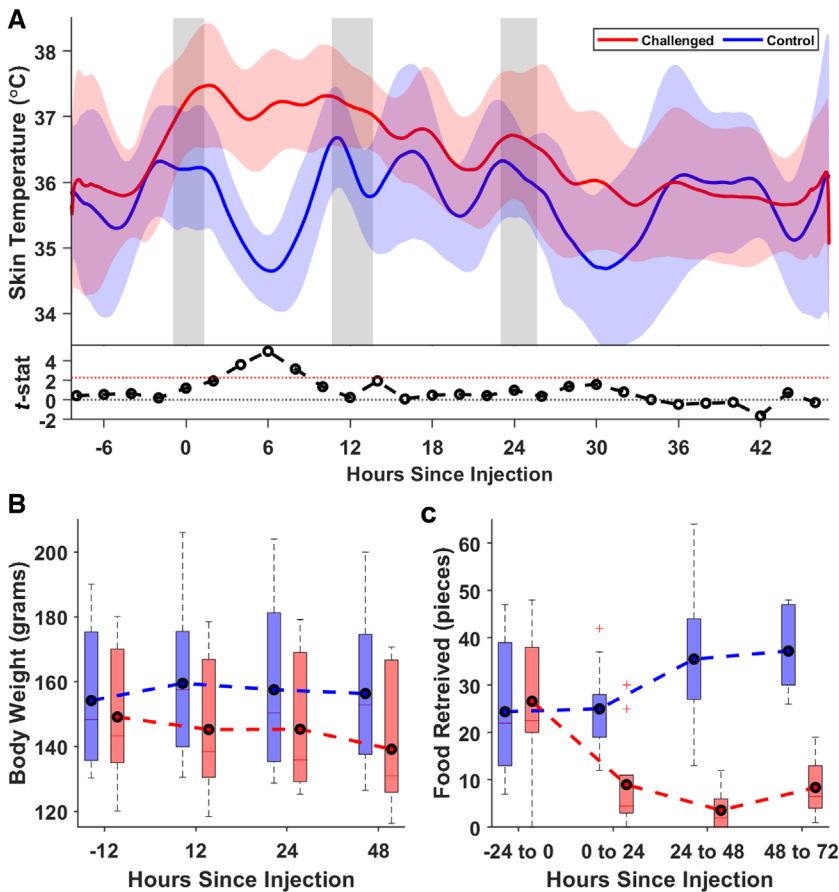
Data were collected pre- and post-injection using a combination of sources. On-animal small biollogger devices (Vesper, ASD inc.) collected body surface temperature (in both closed colony rounds), acceleration (in the second closed colony round), and GPS (in the open colony). Some data were lost because of equipment failure, and data from a sixth challenged bat in the closed colony's second round were discarded due to her death. Infrared cameras continuously tracked food consumption and social isolation. Handling for blood draws and body weight measurements occurred outside the experimental room before and at 12, 24, and 48 h post-injection for the closed colonies and before and 48 h post-injection for the open colony (see Supplementary Methods for full details on data collection and processing, online only). At least three stained blood smears<sup>43</sup> were made for total and differential leukocyte counts, and blood plasma was stored for haptoglobin and lysozyme analysis. Some samples contained insufficient serum for laboratory analysis and had to be excluded. To measure haptoglobin concentration, the standard procedure of the commercial kit "PHASE"<sup>TM</sup> Haptoglobin Assay (Tridelta, Maynooth, Ireland) was followed, matching the use in other bat species.<sup>36,40</sup> To measure lysozyme concentration, we adapted the lysoplate assay to low sample volumes<sup>40,44,45</sup> (see Supplementary Methods for full details on laboratory analyses, online only). Statistical analyses were conducted in SPSS<sup>®</sup> (SPSS Inc, Chicago, IL) and MATLAB (Mathworks, Carlsbad, CA). Generalized linear models were used

to assess changes in proximity to nearest neighbor in both colonies and to assess changes in acceleration in the closed colony and distance flown per night in the open colony. Binomial tests were used to compare the proportion of treatment bats recorded leaving the cluster relative to the proportion of control bats leaving the cluster and to compare the number of instances where the slope of nearest-neighbor records became positive for treatment bats relative to the number of instances in control bats over 320 recorded frames. Mixed ANOVAs were used for the closed colony to assess changes in body temperature, body weight, food consumption, haptoglobin, lysozyme, WBC count, and the neutrophil to lymphocyte ratio (NLR). Paired *t*-tests were used for the open colony to assess changes in body weight, haptoglobin, lysozyme, WBC count, and the NLR. Repeated measures ANOVA was used for the open colony to assess changes in food consumption, while Fisher's exact test was used to compare the proportion of bats that exited the colony at all pre- and post-injection (see Supplementary Methods for full details on statistical analysis, online only).

## Results

### *Evidence of illness response*

The immune-challenged bats showed a clear physiological sickness-like response. Skin temperature of the challenged group in the closed colony was elevated following injection (Fig. 1A;  $F(1,44) = 6.49$ ,  $P = 0.029$  main effect of treatment;  $F(3,44) = 1.17$ ,  $P = 0.33$  main effect of time; and  $F(3,44) = 4.17$ ,  $P = 0.015$  interaction between time and treatment group; mixed ANOVA, with temperature as the response variable, treatment as a between-subjects predictor, and time as a within-subjects predictor). Skin temperature reached its peak at around 80 min after injection at  $37.6 \pm 1.3$  °C (mean  $\pm$  SD). Skin temperature was elevated continuously for at least 8 h post-challenge (Fig. 1A, bottom), with some indications of longer temperature elevation (over 18 h in three individuals, Fig. S1, online only). We observed clear spikes in skin temperature in both the control and challenged groups every time the bats were removed from the colony room for weighing and taking blood samples. We suspect this is due to stress associated with the handling process, as stress has been demonstrated to cause body



**Figure 1.** Evidence of illness response in LPS-challenged bats. (A, top) Skin temperature of challenged bats ( $n = 5$ ) was higher than controls ( $n = 6$ ), mostly in the first 12 hours. Shading for each group's respective mean line shows the 95% confidence interval. Gray shadowed areas depict handling periods, which probably led to a temperature elevation. (A, bottom)  $t$ -statistic for measurements taken every 2 hours. Red dashed line depicts a value of  $t = 2.262$ , which would imply a significant difference at  $P < 0.05$  without corrections. (B) Challenged bats ( $n = 10$ ) lost weight, while controls ( $n = 10$ ) did not. (C) Challenged ( $n = 10$ ) bats, but not control bats ( $n = 10$ ), exhibited anorexia.

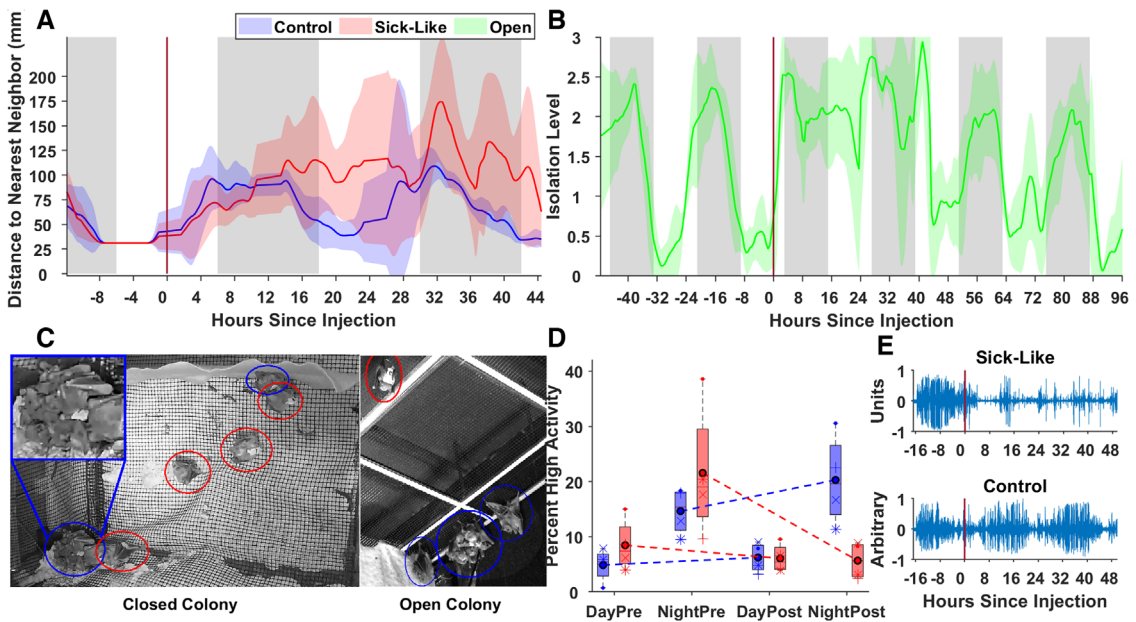
temperature elevation in bats.<sup>46</sup> We did not measure the temperature of free-ranging bats.

In the closed colony, sick-like bats had a significant decrease in weight following LPS injection, losing on average  $9.9 \pm 4.5$  g over 48 h, while control bats had no significant change across time (Fig. 1B;  $F(3,54) = 12.486$ ,  $P < 0.001$  interaction between time and treatment; mixed ANOVA as above, with body weight as the response variable). Moreover, monitoring the food bowl showed that weight loss was at least partially a result of anorexia, as individuals markedly reduced food consumption following LPS injection (Fig. 1C;  $F(1,18) = 36.796$ ,  $P < 0.001$  main effect of treatment;  $F(3,54) = 2.876$ ,  $P = 0.044$  main effect of time; and  $F(3,54) = 12.506$ ,  $P < 0.001$

interaction; mixed ANOVA as above, with the number of pieces of food retrieved as the response variable). Bats in the open colony also lost weight, but the degree of loss was nonsignificant, likely due to the small sample size and the age of these individuals ( $t_4 = 1.854$ ,  $P = 0.137$ ; paired  $t$ -test, with weight as the response variable and time as a within-subjects predictor).

### *Bats isolate themselves when they are sick*

Distance to nearest neighbor (mm) and isolation level (categorical 0–3) in the closed and open colonies, respectively, was significantly explained by the time of day, treatment group, and whether it was pre- or post-LPS injection (Fig. 2A and B; GLMM



**Figure 2.** Sick-like bats exhibit social isolation. Sick-like bats in both the closed (A;  $n = 5$ ) and open (B;  $n = 5$ ) colonies maintained larger distances from neighbors. Nights are indicated by gray vertical shading. Confidence intervals (95%) are shaded for each group's respective line representing the mean. (C) Photos from the closed (left) and open (right) colonies show sick-like individuals (circled in red) out of the bat cluster (greater detail in the top left inset) and distant from conspecifics (circled in blue). Note the single control individual in the closed colony (top right) that perched near sick-like individuals (see Discussion). (D) Challenged ( $n = 4$ ) bats moved much less than control bats ( $n = 4$ ). (E) This is also shown with an acceleration sequence example for the full duration of the study (approximately 64 h) for a challenged bat (top) and a control bat (bottom) with the moment of the treatment marked by a vertical dark red line.

normal distribution with the Laplace fit method (see Table 1 for model components' coefficients and significance). Healthy individuals in both colonies displayed a cyclical pattern of distance from their nearest neighbor following natural circadian rhythms, with the lowest distances occurring during daytime sleep when the bats tightly clustered together and the highest distances during nighttime when they were active. Following LPS injection, sick-like bats in both the closed and open colonies deviated from this pattern and increased distances from the bat cluster during the daytime (Fig. 2C). Interestingly, sick-like bats appeared to isolate themselves from the cluster instead of being rejected by other group members. Video observations revealed that during the initial post-injection rest period, most individuals (4 out of 5 in each round, 8 out of 10 overall) withdrew from the bat cluster (Videos S1–S5, online only), and no treatment individuals joined the cluster upon its formation in the following rest period. This is in contrast with only 3 out of 10 control individu-

als recorded leaving the cluster ( $P = 0.002$ , 9.33 odds ratio of exiting after LPS instead of sham injection, binomial test), and only one control bat stayed out of the cluster for longer than 15 minutes. By analyzing the derivative of the distance to nearest neighbor (Supplementary Methods, online only), we also found that the distance of sick-like bats to their nearest-neighbor began increasing more often than control bats ( $P = 0.004$ , 1.27 odds ratio treatment compared with control, binomial test, counts of positive inflection points out of 320 time points). Moreover, during this isolation period, sick-like individuals displayed dramatically reduced movement, as we quantified with accelerometers attached to the animals in the closed colony (Fig. 2D and E; proportion of time in high activity predicted by between-subjects measure of treatment  $\beta = 0.08$ ,  $P = 0.036$  and within-subjects measures of day/night  $\beta = 0.142$ ,  $P < 0.001$  and whether it was post-injection  $\beta = 0.058$ ,  $P = 0.077$ ; an interaction was found between treatment and day/night  $\beta = -0.127$ ,  $P = 0.002$ . GLMM



**Table 1. Isolation prediction model coefficients**

Predictor	$\beta$	SE	<i>t</i>
<b>Closed colony</b>			
Post-injection***	-77.336	12.141	-6.3699
Treatment***	-65.14	15.543	-4.1909
Time of day***	-30.635	2.6656	-11.493
Post*treatment***	57.945	6.1999	9.346
Post*time***	20.112	2.6922	7.4705
Treatment*time***	14.964	1.6691	8.9653
<b>Open colony</b>			
Nighttime***	0.55816	0.14162	3.9413
Within 2 days of injection***	0.47805	0.1242	3.849
2+ days after injection**	-0.3603	0.11846	-3.0415
Daytime*within 2 days	-0.00612	0.19146	-0.03195
Daytime*after 2 days	0.36659	0.1875	1.9551

\*Asterisks indicate significance as follows: \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , and \*\*\* $P \leq 0.001$ .

normal distribution with the Laplace fit method ( $n = 32$ ). The action of moving out of the cluster for self-isolation was a behavioral exception in light of their general tendency to remain still.

### *Bats stay in when sick*

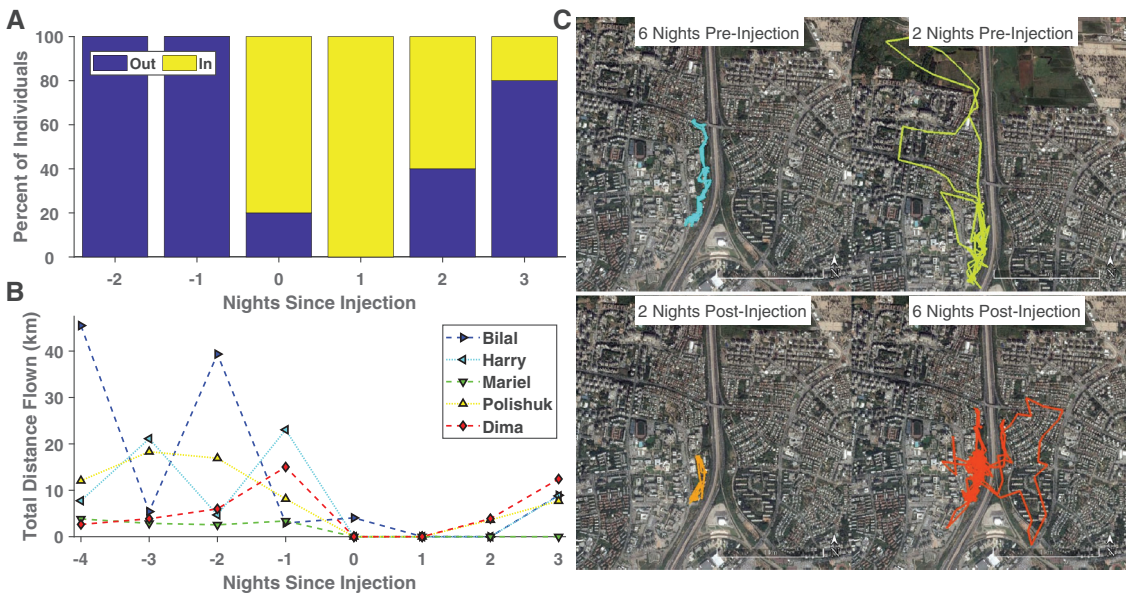
Sick-like bats in the open colony dramatically changed their foraging behavior. Following LPS injection, individuals stayed in the colony for at least two nights and up to five nights, whereas before the injection, they consistently exited to forage (Fig. 2A;  $P < 0.001$ ; Fisher's exact comparing the probability of exiting before and after injection). Moreover, once individuals resumed foraging, they initially flew shorter distances than they had before LPS injection (Fig. 3B and C, and Fig. S5, online only; normalized distance flown predicted by within-subjects measures of night  $\beta = 0.005$ ,  $P = 0.984$  and treatment  $\beta = -2.962$ ,  $P = 0.004$ . GLMM normal distribution with the Laplace fit method  $n = 40$ ). We validated that the staying in behavior was not an artifact of the food supplement that was given in the open colony by showing that these bats ate significantly less than expected given their body weight (Fig. S6, online only;  $F(2,14) = 8.205$ ,  $P = 0.012$ ; post-hoc:  $P = 0.012$  night 1 to expected,  $P = 0.044$  night 2 to expected; repeated ANOVA, with the number of pieces of food retrieved as the response variable and night at the within-subjects predictor). The decrease in food consumption was similar in the two colonies (bats consumed  $23.39 \pm 24.83\%$  vs.

$49.87 \pm 29.6\%$  of the expected amount in the closed and open colonies, respectively).

### *APR in fruit bats*

Immunologically, there was a clear APR as demonstrated by multiple blood parameters. Total WBC count showed no significant difference between challenged and control groups at all times in both the closed and open colonies. However, the NLR, a ratio of the count of the two WBC types most involved in the APR that also serves as an indicator of physiological stress, was found to be significantly higher in the treatment group following LPS injection for the closed colony (Fig. 4A;  $F(3,43) = 5.068$ ,  $P = 0.007$  interaction between treatment and time; mixed ANOVA as above, with NLR as the response variable). In the closed colony, the challenged group maintained a similar NLR throughout the experiment, while the control group's ratio decreased after the initial measurement. The NLR was higher before LPS injection in the open colony ( $t_4 = -4.118$ ,  $P = 0.015$ ; paired  $t$ -test as above, with NLR as the response variable).

Haptoglobin concentration was significantly higher following LPS injection (Fig. 4B). In the closed colony, haptoglobin was significantly higher after injection in the challenged group than the control ( $F(3,71) = 41.716$ ,  $P < 0.001$  interaction between treatment and time; mixed ANOVA as above, with haptoglobin as the response variable) particularly after 24 and 48 h (24 h post:  $1.17$  mg/mL compared with  $6.73$  mg/mL,  $P = 0.004$ ;



**Figure 3.** Sick-like bats stop foraging. Following LPS injection, individuals ( $n = 5$ ) in the open colony (A) did not exit to forage, shown in yellow, and (B) flew shorter distances on their first trip back out than on trips before injection. This can also be seen through GPS tracks of one individual (Polishuk) with examples (C) before injection (top, in blue and yellow), on the first night out after injection (bottom left, in orange), and after recovery (bottom right, in dark orange).

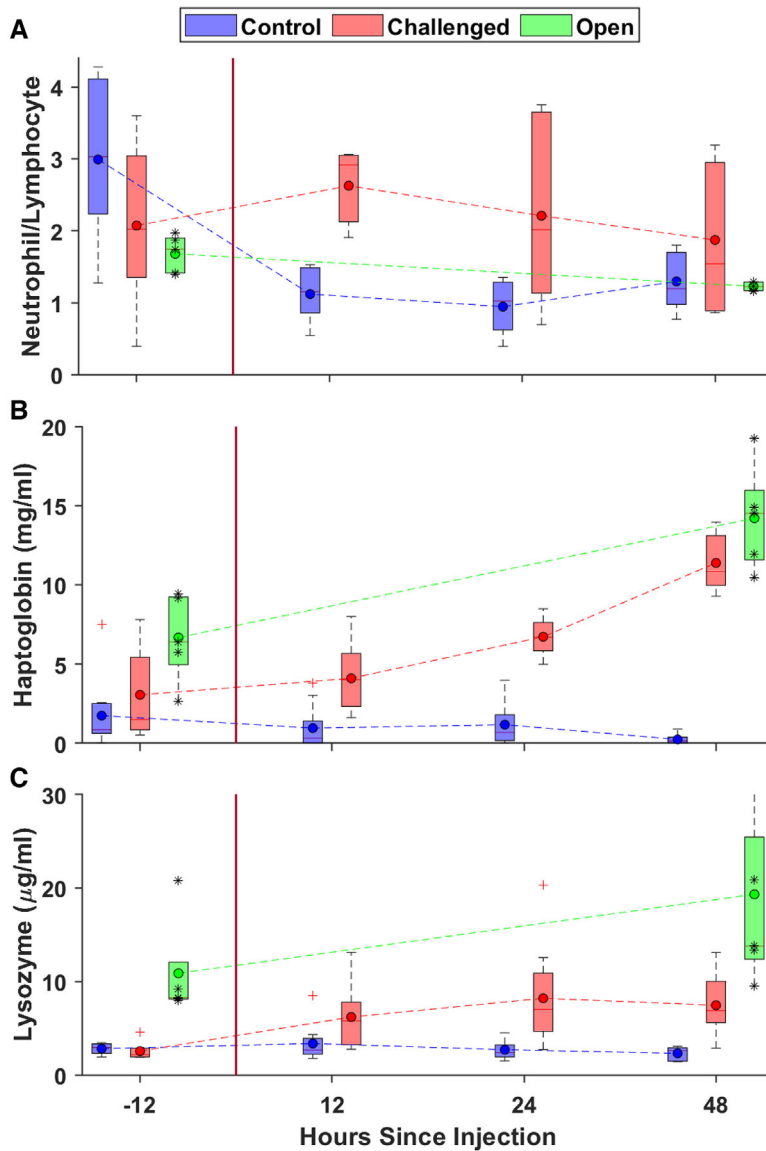
48 h post: 0.23 mg/mL compared with 11.38 mg/mL,  $P < 0.001$ . Averages for the control and challenged groups, respectively). In the open colony, the young adults had higher haptoglobin levels after 48 h than before LPS injection ( $t_4 = 5.22$ ,  $P = 0.006$ ; paired  $t$ -test as above, with haptoglobin as the response variable). Lysozyme concentration was higher following LPS injection in the experimental group in the closed colony but not in the open colony (Fig. 4C;  $F(3,70) = 9.087$ ,  $P < 0.001$  interaction between treatment and time; mixed ANOVA as above, with lysozyme as the response variable;  $t_4 = 1.352$ ,  $P = 0.25$ ; paired  $t$ -test as above, with lysozyme as the response variable; respectively).

## Discussion

Egyptian fruit bats present a valuable opportunity to understand sickness responses in long-lived, highly social mammals that live in densely populated, high-contact groups. We sought to understand the behavioral and physiological response to bacteria, a common yet relatively understudied cause of illness, in *R. aegyptiacus* and other bat species. We used a standard immune challenge in order to induce an APR of the innate immune system and the accom-

panying sickness behavior. Despite using a relatively medium dosage of LPS compared with similar experiments in other bat species,<sup>30,32,33,40</sup> our bats were very clearly sick. LPS-challenged bats had fever, lost weight, consumed less food than before the challenge, and developed clear sickness behavior, including lethargy, reduction in social interaction, and general movement.

Skin temperature was found to be a reliable proxy of body temperature, as the values measured prechallenge are known to fit normal healthy fruit bats.<sup>47</sup> Body temperature elevation in response to an immune challenge is observed across bat species, as it is in other mammals, although Melhado *et al.*<sup>34</sup> point out this only occurs in bats challenged during the resting phase,<sup>28–30,32</sup> and not for bats challenged during the active phase.<sup>31,34</sup> We challenged bats in both active and resting phases, although we did not have a large enough sample to compare between the two. The observed temperature elevation in our study of 1–1.5 °C is similar to that found in other bat species, including *C. perspicillata*<sup>32</sup> and *A. lituratus*.<sup>30</sup> *Rousettus* elevated fever was maintained for over 8 h post-challenge, longer than reported for other bat species (up to 6 h), although a few individuals showed elevated temperature for



**Figure 4.** Immunological responses. Changes in (A) the neutrophil to lymphocyte count ratio (red, challenged,  $n = 6$ ; blue, control  $n = 6$ ; and light green, open  $n = 5$ ), (B) haptoglobin concentration, and (C) lysozyme concentration in both the closed (red, challenged,  $n = 10$ ; and blue, control,  $n = 10$ ) and open (light green,  $n = 5$ ) colonies.

much longer periods. Notably, the behavior sickness affects lasted longer than the fever for all bats.

During sickness, bats isolate themselves and hang apart from conspecifics either by failing to join a cluster during its formation or by actively leaving the cluster during daily sleeping hours (see Supplementary Videos, online only). Perching alone is extremely atypical for this species. This behavior was initiated spontaneously by the sick-like bat and

did not appear to be a response to any behavior of the healthy controls, although this possibility could not be ruled out based on the current study. Sick-like individuals then maintained their isolation for about 2 days. While general listlessness during illness, which we could accurately estimate using acceleration measurements, clearly contributes to distance from conspecifics, particularly during the sleeping hours of the second day, multiple instances



of individuals leaving the cluster or changing locations within the room from one isolated location to another demonstrate this is not the sole explanation. To our knowledge, this is the first documented instance detailing a sick mammal actively moving away from a social group, although it is probably not unique to *R. aegyptiacus* and remains to be described in other species. Such self-isolation behavior is notably different from what has been observed in other nonhuman mammals. Despite social withdrawal being considered a hallmark of sickness behavior,<sup>3</sup> previous findings of mammalian social isolation are of healthy individuals avoiding sick conspecifics,<sup>15,48,49</sup> or sick individuals reducing interactions<sup>41,50</sup> or shared space use<sup>24</sup> because of lethargy. Interestingly, we observed one healthy female that consistently approached sick-like isolated females, which she had been housed with for months, and hung near them. While this is only a single anecdote, it is the exact opposite of what we would expect if healthy individuals were avoidant in order to protect themselves from catching a disease. Additionally, it indicates the possibility of a role of social history or demographics in moderating behavior between healthy and sick individuals, such as in vampire bats.<sup>41</sup>

Removal from the cluster may directly benefit the infected individual in multiple ways. First, hanging in a less insulated location may keep an individual's fever from becoming dangerously high. Alternatively, moving outside of the bat cluster might instead incur a health cost as it sacrifices the thermal benefits of clustering.<sup>51</sup> More research is needed to tell these two options apart. Isolation behavior contrasts with the behavior of free-ranging kudu, where sick individuals sought warm environments to support the febrile response.<sup>22</sup> Interestingly, the timing of body temperature increase and cluster removal are offset from one another, indicating some behavioral lag, with the earliest cluster-exiting event occurring later than the beginning of the temperature increase (about 2.5 h after injection) and social isolation effects continuing through the next sleeping phase when temperature differences are less clear. Some of these connections may have also been obscured due to the timing of LPS injection and the natural cycles of healthy bats, as the beginning of the active period (when the control bats were more spread out) overlapped with the 6–8-h post-injection window when the temperature dif-

ferences were the greatest, potentially hiding isolation effects in the sick-like bats during this time. Second, bats within clusters frequently squabble and push one another.<sup>52</sup> By not being in the cluster, sick individuals would not have to expend energy on interindividual conflict or maintaining cluster position. If true, this would explain why the self-removal behavior was observed in this species and has not been documented in other social species who do not sleep in such high-density conditions. Finally, self-isolation may prevent an already sick individual from catching an additional illness, as multiple concurrent illnesses are far more deadly than lone infections.<sup>2</sup>

While most likely driven by benefits to the individual, this behavior may serve to reduce transmission and benefit the group as a byproduct. Previous analysis suggests that the roostmates of Egyptian fruit bats are not more related to one another than would be expected by chance,<sup>53</sup> making kin selection an unlikely evolutionary driver, but group selection might still play a role. This emphasizes the importance of byproduct benefits in the evolution of sickness behavior. Like humans reducing contacts when sick<sup>54</sup> or social distancing efforts made in 2020 to slow the spread of COVID-19,<sup>55</sup> increasing space between individuals may reduce infection rates, particularly in densely populated groups, such as the ones Egyptian fruit bats live in.

This study is the first to record changes in foraging of sick-like bats in the wild at the individual level. All sick-like individuals in the open free colony failed to exit to forage for at least two nights. This was clearly not exclusively a result of the food supplement as these bats always flew out to forage before the treatment and as they consumed little food during the treatment, similar to the decrease in food consumption observed in the closed colony. While we cannot exclude the possibility that fully wild bats may engage in short flights while sick, the clear pattern of well-known sickness behaviors, such as anorexia and extreme lethargy, due to the APR of the immune system, documented here, makes this highly unlikely. These findings demonstrate the lack of food consumption widely observed in laboratory and livestock settings<sup>3,56–59</sup> also occurs in free-ranging animals, supporting the highly conserved presence of anorexia in sick animals. As our bats in the open colony exhibit foraging behavior very similar to other wild bats,<sup>60</sup> we

predict that this behavior is general for other bat species as well. In the wild, foraging can take considerable effort and exposes animals to predation risk, particularly for individuals that are not in good condition. Thus, staying in place and not traveling to forage benefits sick animals by conserving energy and increasing safety. Anorexia is also thought to support the immune response to bacteria,<sup>61,62</sup> and laboratory studies in mice have demonstrated increased survival in individuals with restricted food intake either before<sup>62</sup> or during<sup>63</sup> infection. Finally, it may also reduce disease transmission by decreasing the number of contacts between sick individuals and shared food sources.<sup>7</sup>

The APR has been examined in six species of bats, revealing much variability. Some show clear leukocytosis (*Chaerephon plicatus*<sup>64</sup> and *D. rotundus*<sup>33</sup>), but in others, this response is unclear (*A. lituratus*<sup>30</sup>), totally absent (*M. molossus*<sup>31</sup>), or present in some scenarios (*C. perspicillata*<sup>32</sup>) but not in others (*C. perspicillata*<sup>29,34</sup>). We did not find leukocytosis. Normal, healthy captive *R. aegyptiacus* display an extremely wide range in leukocyte count (see Abdel-Rachied *et al.*<sup>65</sup>), making it difficult to follow leukocytosis even if leukocytosis did occur. We believe that a significant leukocytosis in the challenged group might have been masked by a stress leukogram (also known as NLR, see Davis *et al.*<sup>66</sup>) that was caused by the handling of bats from both groups, reflected in elevation of neutrophil and decrease in lymphocyte counts in the blood sample before the challenge. Stress leukogram was found to maintain the higher ratio in the challenged group, similar to previous findings in *D. rotundus*<sup>33</sup> and food-deprived *C. perspicillata*<sup>29</sup> in reaction to LPS challenge.

Molecularly, we are the first to measure lysozyme and the second to measure haptoglobin in bats during a bacterial immune challenge. Haptoglobin has only been measured in a few bats, which showed a clear elevation in response to bacterial,<sup>67</sup> fungal,<sup>36</sup> and human stress.<sup>40</sup> In both free and captive bats, we observed a clear, continuous increase in haptoglobin during the entire period of the study, as also observed in other mammals.<sup>68</sup> It is interesting to see that even at baseline, some individuals' haptoglobin level was not zero, perhaps due to the stress of handling. The free-ranging colony's higher baseline levels could have been due to the younger age

of these bats or their potential nightly exposure to bacterial pathogens outdoors.

Lysozyme levels were higher following LPS injection in the experimental group in the closed colony but not in the open colony. This finding has several potential explanations. First, the open colony was examined only before and 48 h after challenge, which was later than the peak in lysozyme levels at 24 h post-challenge in the closed colony; thus, the lysozyme levels may have decreased by that point. Second, there may be an age difference between adults and adolescents in the lysozyme level, as was found in bison.<sup>69</sup> Third, the relatively small number of bats examined in the open colony and high variation between individuals may have obscured any potential effect.

## Conclusion

We demonstrate that in addition to showing the classical mammalian sickness physiological response, sick-like Egyptian fruit bats exhibit voluntary, apparently self-imposed, social distancing. This is the first time that social self-isolation has been recorded in a mammal, rather than isolation through avoidance by conspecifics or as a byproduct of lethargy. Such isolation behavior stands in stark contrast to the normal behavior in this species, reflecting a temporary shift in the need to prioritize survival. Moreover, anorexia, a well-documented aspect of sickness behaviors, is also documented in the context of staying in the sleeping shelter, as expected based on our current understanding of the role of anorexia during bacterial infection. The bats' self-isolation together with their staying in the colony rather than foraging outdoors suggests how sickness behavior can reduce the transmission of pathogens both inside and outside the colony, including reducing the probability of inter-species spillover events. This supports previous findings that spillover events are primarily caused by human disturbance,<sup>70</sup> emphasizing the importance of leaving critical habitat features, such as roosts, alone to reduce the likelihood of future events like the COVID-19 pandemic.

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### Author contributions

M.W., G.Á.C., Y.Y., and L.G.H.M. conceived of and designed the overall study. K.R.M. conceived of and designed the social behavior component of the study. L.H. conceived, designed, and implemented the GPS tracking component of the study. M.W., K.R.M., and V.B.S.R. conducted closed colony data collection. M.W., L.H., and K.R.M. conducted open colony data collection. M.W. and G.Á.C. conducted laboratory analyses on immunological measures. K.R.M. conducted statistical analyses. K.R.M. and M.W. drafted the manuscript (with edits from Y.Y. and G.Á.C.). All authors contributed to and approved of the final manuscript.

### Ethics approval

All applicable institutional and/or national guidelines for the care and use of animals were followed. Animal care and experimental procedures were approved by Tel Aviv University IACUC under approval form ID 04-19-002.

### Availability of data and materials

The dataset supporting the conclusions of this article is available as a Supplementary Information File, online only.

### Supporting information

Additional supporting information may be found in the online version of this article.

**Video S1.** Two bats removing themselves from the social cluster, wide view.

**Video S2.** One bat removing itself from the social cluster, wide view.

**Video S3.** Two bats removing themselves from the social cluster, close view.

**Video S4.** One bat removes itself from the social cluster, close-up view.

**Video S5.** Video of the cluster showing an active control bat and a sick-like bat removing itself from the cluster.

**Figure S1.** Skin temperature of challenged (red) and control (blue) bats with solid lines for the group average and dotted lines for each individual.

**Figure S2.** Distance from nearest neighbor is shown for control (blue) and sick-like (red) bats in the closed colony.

**Figure S3.** The isolation level is shown for bats in the open colony.

**Figure S4.** The proportion of time in high activity, based on accelerometry values, for individuals (dotted lines) and group averages (solid lines) is shown for the control (blue) and sick-like (red) bats.

**Figure S5.** GPS tracks for two individuals.

**Figure S6.** Individuals ate less than expected (shown as a black line) during their first two nights staying inside the colony).

Supplementary Material

### Competing interests

The authors declare no competing interests.

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