

Brief communication

Prolonged separation delays wound healing in monogamous California mice, *Peromyscus californicus*, but not in polygynous white-footed mice, *P. leucopus*

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Abstract

Social interactions are often stressful, but under certain circumstances, they may be beneficial for health and well-being. In a previous study, wound healing was slowed after mate separation (2 days) in monogamous California mice, *Peromyscus californicus*, but not polygynous white-footed mice, *P. leucopus*. Although these results indicate that positive social interaction is critical for immune activity in some species, the extent to which such social effects are enduring remains unspecified. The goal of the present experiments was to determine whether a period representing ~20% of expected adult lifespan of these species in the wild (8 weeks) would affect wound healing. Because our experimental design required that the same animals were wounded twice, we were also able to determine the extent to which wound healing is repeatable. Wound healing remained delayed after 8 weeks of separation in *P. californicus*, and healing scores were not correlated between first and second wounds within individuals. In *P. leucopus* however, housing conditions did not influence wound healing, but first and second wound healings were correlated indicating repeatability. In sum, our results suggest that positive social interactions may be important for promoting immune activity in some species. © 2006 Elsevier Inc. All rights reserved.

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1. Introduction

Social interactions can have positive or negative consequences, but the majority of studies have focused on antagonistic types of relations among individuals. Presumably, this focus has been driven by the recurrent observation that negative social interactions are often stressful. Social antagonism often elevates the activity of the hypothalamic–pituitary–adrenal axis (HPA) [1] and leads to increased production of glucocorticoids, the vertebrate “stress” hormones [2], although this result is not uniform across all species [3]. Occupation of a subordinate position within a social hierarchy has similar consequences; low ranking animals generally exhibit higher baseline concentrations of glucocorticoids than their high-ranking counterparts [4, 5]. As with social antagonism though, this outcome is

context-dependent and often the stability of the social system determines if and when animals are more stressed [6].

Experiences of strong social stressors, especially over long periods, can detrimentally affect multiple aspects of vertebrate physiology and behavior [2]. One well-studied consequence of social stressors is suppression of immune activity. For example, mice exposed to chronic social stressors increased concentrations of glucocorticoids and subsequent reactivation of latent herpes simplex viral infections [7]. Sometimes, even weak negative social interactions can have pervasive effects on the immune system. For instance, perception of increased population density compromised immune activity in prairie voles (*Microtus ochrogaster*) [8]. Although stressful social interactions typically compromise immune activity, affiliative social interactions may enhance it. Mounting evidence indicates that some social interactions can reduce stress [9,10], which may improve immune activity. For example, group housing of prairie voles (but not meadow voles; *M. pennsylvanicus*) improved

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lymphocyte proliferation to mitogen stimulation [11]. Similarly, female Siberian hamsters (*Phodopus sungorus*) housed with their cage-mates showed increased rates of wound healing compared to single-housed individuals [12].

Recently, the effects of social housing on rate of wound healing were examined in three species of *Peromyscus*, each with a different reproductive strategy [13]. Monogamous *Peromyscus californicus* and facultatively monogamous *P. eremicus* separated from cage-mates for 2 days delayed wound healing compared to mice housed in pairs before and throughout the healing process. Wound healing in a polygynous related species, *P. leucopus*, however, was unaffected by housing conditions. In the present study, we hypothesized that wound healing would continue at a slow rate when mice were separated from their cage-mates for long periods of time. Specifically, we sought to determine whether 8 weeks of separation would i) continue to delay wound healing in *P. californicus*, and ii) affect rate of wound healing in *P. leucopus* at all. We expected that wound healing in *P. californicus* would remain delayed after prolonged isolation. In *P. leucopus*, we expected that even long-term social separation would not affect rate of wound healing because of the lack of strong social affiliation in this species.

2. Methods

2.1. Animals

All procedures herein were conducted in accordance with the *National Institutes of Health Guidelines for the Care and Use of Laboratory Animals*. Prior to experiments, our protocol was approved by the Institutional Animal Care and Use Committee of The Ohio State University. *P. californicus* and *P. leucopus* used in experiments were procured from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC, USA). Upon arrival at our animal facility at The Ohio State University, adult male experimental mice (>60 days of age) were housed either singly or in pairs, maintained on a 14L:10D light cycle at 22.5 (\pm 1) °C, and given food (TekLad 8620) and tap water ad libitum for two weeks prior to the start of the experiment.

2.2. Experimental procedure

2.2.1. Experiment 1

Male *P. leucopus* ($n=6$) and *P. californicus* ($n=5$) were wounded while housed with a conspecific (paired). Animals were anesthetized with isoflurane in O₂ enriched-air and a patch of fur (approx. 90 mm²) was shaved on the dorsum between the scapulae. This shaved region was sterilized with 70% ethyl alcohol, and two circular wounds (3.5 mm in diameter) were made in the dorsal skin using a sterile, disposable biopsy punch tool (Miltex Instrument, Bethpage, NY, USA). Wound size was then measured over the following 8 days (see below). At the end of this period, cage-mates were removed; 8 weeks later, the same experimental mice received a second wound. At the end of the second wound measurement period, all mice were euthanized via CO₂ inhalation. Due to the limited numbers of

mice available from the *Peromyscus* Genetic Stock Center at the time, the gender of cage-mates in pair-housed animals during the study was variable; data indicate that the sex of the cage-mate does not dramatically alter rate of wound healing in these species however (Glasper and DeVries, unpublished data). Experimental animals however (i.e. wounded animals) were exclusively male.

2.2.2. Experiment 2

In this part of the study, *P. californicus* ($n=5$) were housed with conspecifics for 14 days, then they were wounded as described above and wound size was measured for 8 days. Eight weeks later, experimental mice were wounded again, and wound size measured for the following 8 days. In this experiment, cage-mates were not removed from cages. *P. leucopus* were not included in this part of the study because preliminary analysis of data from Experiment 1 indicated no effect of housing conditions on wound healing.

2.3. Wound measurement

Immediately following wounding and each day for 7 days thereafter (between 1000 and 1400h), wounds were photographed using a digital camera (Coolpix 775, Nikon Tokyo, Japan). A reference standard (a 3.5 mm inner diameter circle on a white background) was included in every photograph. In each photo, entrance wounds and reference standards were traced and areas were calculated using graphic design software (Canvas 6, Deneba Systems, Miami, FL, USA). The ratio of the wound area to the reference standard was then calculated for each photograph. During these measurements, mice were handled and anesthetized comparably. Wound size reduction (wound healing) was calculated each day by dividing the standardized wound size by the standardized area on day 0. Oftentimes, wound size was >1 because of swelling, presumably due to local inflammation. Finally, to ensure that the wounding or photographing process did not affect the condition of mice, body mass was measured each day just prior to photographing.

2.4. Statistical analysis

Wound size distributions across days and groups did not depart significantly from normal according to 1-sample Kolmogorov–Smirnov tests. Wound healing (% of initial wound size, adjusted to reference standard) was compared between housing groups using repeated-measures general linear models with wound size and housing conditions as within group factors (when the same individuals were wounded twice), or second wound healing profiles were compared between pair-housed and single-housed *P. californicus* with wound size as within-group factors and housing as between group factors (when mice were wounded twice, but the second wound healing profiles were compared between distinct groups of mice). The slope of healing curves was also calculated for each individual over the first three days of the healing process of first and second wounds; these values were then compared using paired *t*-tests.

Because first wounds were produced by one investigator (E.G.) and second wounds were made by a different investigator (L.M.), all wounds in Experiment 1 were scored blind to treatment group by both investigators and directly compared using repeated-measures GLM. Scorer identity did not significantly affect wound size estimates ($p>0.05$); subsequently, this factor was not included in analyses. Additionally, scoring of wound size late in the healing process was somewhat unrepeatable. Each wound photo was scored three times then coefficients of variation (CV) were calculated for each wound. CV's changed over the course of the wound healing process ($F(1, 11)=52.8, p<0.05$) and became significantly different between species late in the response (day 8; between time point difference identified by Bonferroni post hoc test). For this reason, only wound scores from day 0–7 were used in the following analyses. Presumably, increased measurement error late in the response is due to the formation of scabs at this time point in these species, which makes the visual scoring process on or after this point unreliable. All statistical tests were conducted with SPSS v.12, setting $\alpha=0.05$.

3. Results

Rate of wound healing of first versus second wounds was not different in *P. leucopus*. Wounds healed significantly over time in all animals (Fig. 1A; $F(6, 30)=12.7, p<0.05$), but whether wounds were administered once or twice did not affect the healing process ($p>0.05$). The lack of differences ($p>0.05$) in slopes of healing curves over the first three days of healing further supported this lack of difference in healing in *P. leucopus*. First and second wound healing slopes were significantly correlated, however ($\beta=0.82, p<0.05$), indicating that wound

healing is a repeatable measure in this species. Body mass did not change significantly over the course of wound healing in either species ($p>0.05$).

In *P. californicus*, wound healing tended to differ between first and second wounds. Wounds healed over the course of the experiment (Fig. 1B; $F(6, 24)=7.06, p<0.05$), but first wounds (in pair-housed mice) tended to heal faster than second wounds (in separated mice: $F(1, 4)=5.4, p=0.08$). This difference was not reflected in comparisons of the slopes of healing curves during the first three days of healing however ($p>0.05$). Also, in contrast to *P. leucopus* above, healing slopes of first and second wounds were not correlated in *P. californicus* ($p>0.05$).

To determine whether differences in healing profiles between first versus second wounds in *P. californicus* was due to persistent effects of separation or to the number of wounds experienced, we compared rate of first versus second wound healing in an additional group of *P. californicus* housed with cage-mates prior to and for the duration of first and second wound healing process. In this case, wound size changed over time (Fig. 1C; $F(6, 24)=22.0, p<0.05$), but there was no significant difference between first and second wound healing profiles ($p>0.05$). Similarly, slopes of healing curves during the first three days of healing did not differ ($p>0.05$) and were not correlated ($p>0.05$). Finally, to determine whether separation specifically affected wound healing rate, we compared second-wound healing profiles between pair-housed and separated mice. Pair-housed *P. californicus* healed significantly more quickly than separated individuals (Fig. 1D; $F(1, 8)=45.9, p<0.05$), even though both had been wounded previously. However, comparisons of healing slopes for the first three days post-wounding did not identify an effect of housing conditions ($p>0.05$).

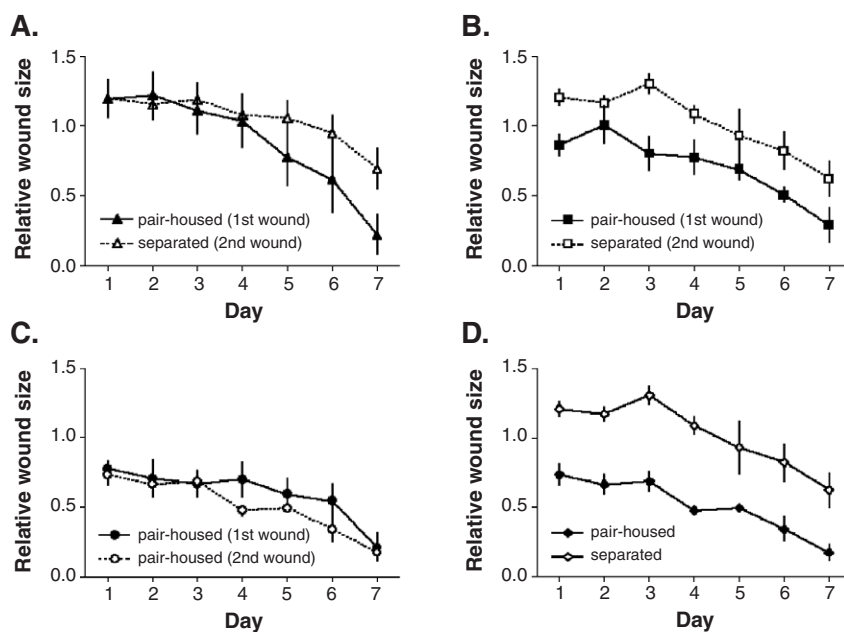


Fig. 1. Wounds heal more slowly in *Peromyscus californicus*, but not *P. leucopus*, separated from cage-mates for 8 weeks. A) Comparison of wound healing in pair- (1st wound) versus single-housed (2nd wound) *P. leucopus*; B) comparison of wound healing in pair- (1st wound) versus single-housed (2nd wound) *P. californicus*; C) comparison of wound healing in pair- (1st wound) versus pair-housed (2nd wound) *P. californicus*; D) comparison of wound healing in single- (2nd wound) versus pair-housed (2nd wound) *P. californicus*. Bars represent means \pm SEM.

4. Discussion

Social stressors can suppress immune activity [14,15]. Establishing a new position in a dominance hierarchy, experiencing an unfamiliar environment, or being separated from mates, relatives or parents can increase circulating corticosteroids [1,9], which can lead to reduced immune responsiveness [2,16]. Social interactions are not obligatorily immunosuppressive though; positive social interactions can reduce circulating corticosterone [9,17], which sometimes enhances immune activity [11,18]. For instance, undergraduate students with strong feelings of social support had enhanced responses to (tertiary) herpes vaccinations [19]. Likewise, social support ameliorated brain damage associated with stroke [20].

Our central interest in this study was to determine whether a lack of positive social stimulation could also affect immune activity in *Peromyscus* mice when animals were isolated for an extended period of time. In a previous study, wound healing was delayed in *P. californicus* separated from mates for 2 days, but *P. leucopus* were not affected by a similar treatment [13]. Here, the effects of separation persisted for 8 weeks post-separation in *P. californicus* whereas wound healing in *P. leucopus* remained unaffected by isolation even over this extended period. Studies in humans report that separation can have dramatic long-term consequences on health. Separated or divorced individuals, for instance, display a 6-fold higher mortality rate to pneumonia than married individuals [21]. Sometimes, even the lack of a feeling of social support can reduce measures of immune activity [22]. The results of our study illuminate the effects of separation on one integrative measure of immune activity. Rate of wound healing is contingent on the function of multiple types of immune cells, as well as the cytokines they secrete and the repair cells these substances recruit to the site of wounding [7,15]. Moreover, rate of wound healing is easily interpretable in a functional sense — more rapid healing is critical to reduce the likelihood of bacterial infection [23].

The presence of an effect of separation in *P. californicus*, but not *P. leucopus* in our study is probably related to the unique social system of each species. *P. californicus* are monogamous [24], whereas *P. leucopus* are polygynous [25]. Typically, lack of affiliative stimulation is more stressful in species that maintain long-term pair bonds [26]. Subsequently, absence of social stimulation may only affect wound healing in the species that would normally experience some form of social support in the wild. Still, the persistence of retarded wound healing in *P. californicus* after 8 weeks of separation is somewhat counterintuitive. Assuming that individuals of this species live about 1 year in the wild [27], 8 weeks represents almost 20% of adult life for this species (assuming maturity occurs at 77 days) [28]. Over this period, one might expect that mice would have adjusted to social isolation because selection against slow-healing phenotypes would likely be intense. If so, one would predict that wild *P. californicus* must rarely remain alone in the wild.

In addition to differential effects of separation on wound healing, the repeatability of wound healing varied between

species. First and second wound healing rates were correlated in *P. leucopus*, but not in *P. californicus*. When developing our experimental protocol, we expected that second wounds would heal more slowly in both species because immune function is often energetically expensive [29,30]. In other words, we hypothesized that wound healing might generate a resource deficit, which would impinge on the quality and/or rapidity of a second wound healing process. Although there was no evidence for such a trade-off here, it was clear that wound healing was more labile in *P. californicus* than *P. leucopus*. The reason could lie in the variable concentrations of basal and stress-induced corticosterone between species and their effects on wound healing [13]. Previous work demonstrated that restraint stress does not increase circulating corticosteroids or improve wound healing in *P. californicus*, but does in *P. leucopus* [13]. Other studies have shown that similar phenomena exist even between populations of the same species [31].

Although our work demonstrates that social separation can have enduring effects on wound healing, corticosterone was not measured so its influence on rates of wound healing is unknown in our study. *P. californicus* separated from cage-mates for long periods of time may have higher corticosterone titers than their paired conspecifics, but this hypothesis remains to be tested directly. Previous work, however, indicates that differences in baseline and stress-induced corticosterone titers cannot explain separation effects on wound healing in *P. californicus* as they do in *P. leucopus* [13]. In Siberian hamsters (*P. sungorus*), another putatively monogamous species [32], isolation did not increase baseline corticosterone, but it did delay wound healing. Finally, in a different study on *P. leucopus*, pair-housing generally decreased corticosterone concentrations, but it also reduced immune responsiveness in the same animals [33]. Considered together, it seems that i) corticosterone must not be the main mediator of separation-induced changes in immune activity in *P. californicus*, and ii) the effects of separation induced changes in corticosterone in some species may be different depending on the type of immune activity measured.

In addition to continuing efforts to identify the underlying mechanisms of separation-induced delays in wound healing, future work should also consider the significance of the relatedness and/or age of the cage-mate on healing profiles [34]. Indeed, it would be intriguing to contrast rates of wound healing in males versus females in our experimental paradigm, as data from humans suggest that women typically do not adjust (immunologically) to separation as rapidly as men [35].

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References

- [1] Tamashiro KKK, Nguyen MMN, Sakai RR. Social stress: from rodents to primates. *Front Neuroendocrinol* 2005;26:27–40.
- [2] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 2000;21:55–89.
- [3] Marler CA, Oyegbile T, Plavicki J, Trainor BC. Response to Wingfield Commentary on “A continuing saga: the role of testosterone in aggression”. *Horm Behav* 2005;48:256–8.
- [4] Virgin CE, Sapolsky RM. Styles of male social behavior and their endocrine correlates among low-ranking baboons. *Am J Primatol* 1997;42:25–39.
- [5] Stavisky RC, Adams MR, Watson SL, Kaplan JR. Dominance, cortisol, and behavior in small groups of female cynomolgus monkeys (*Macaca fascicularis*). *Horm Behav* 2001;39:232–8.
- [6] Abbott DH, Keverne EB, Bercovitch FB, Shively CA, Mendoza SP, Saltzman W, et al. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Horm Behav* 2003;43:67–82.
- [7] Padgett DA, Marucha PT, Sheridan JF. Restraint stress slows cutaneous wound healing in mice. *Brain Behav Immun* 1998;12:64–73.
- [8] Nelson RJ, Fine JB, Demas GE, Moffatt CA. Photoperiod and population density interact to affect reproductive and immune function in male prairie voles. *Am J Physiol, Reg* 1996;270:R571–7.
- [9] DeVries AC, Glasper ER, Detillion CE. Social modulation of stress responses. *Physiol Behav* 2003;79:399–407.
- [10] Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R. Emotions, morbidity, and mortality: new perspectives from psychoneuroimmunology. *Annu Rev Psychol* 2002;53:83–107.
- [11] Klein SL, Hairston JE, DeVries AC, Nelson RJ. Social environment and steroid hormones affect species and sex differences in immune function among voles. *Horm Behav* 1997;32:30–9.
- [12] Detillion CE, Craft TKS, Glasper ER, Prendergast BJ, DeVries AC. Social facilitation of wound healing. *Psychoneuroendocrinology* 2004;29:1004–11.
- [13] Glasper ER, DeVries AC. Social structure influences effects of pair-housing on wound healing. *Brain Behav Immun* 2005;19:61–8.
- [14] Padgett DA, Glaser R. How stress influences the immune response. *Trends Immunol* 2003;24:444–8.
- [15] Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. *Nat Rev, Immunol* 2005;5:243–51.
- [16] deGroot J, Boersma WJA, Scholten JW, Koolhaas JM. Social stress in male mice impairs long-term antiviral immunity selectively in wounded subjects. *Physiol Behav* 2002;75:277–85.
- [17] DeVries AC, DeVries MB, Taymans S, Carter CS. Modulation of pair bonding in female prairie voles (*Microtus ochrogaster*) by corticosterone. *PNAS* 1995;92:7744–8.
- [18] Kiecolt-Glaser JK. Stress, personal relationships, and immune function: health implications. *Brain Behav Immun* 1999;13:61–72.
- [19] Glaser R, Kiecolt-Glaser JK, Speicher CE, Holliday JE. Stress, loneliness, and changes in herpesvirus latency. *J Behav Med* 1985;8:249–60.
- [20] Hattori K, Lee H, Hurn PD, Crain BJ, Traystman RJ, DeVries AC. Cognitive deficits after focal cerebral ischemia in mice. *Stroke* 2000;31:1939–44.
- [21] Verbrugge LM. Sex differences in health. *Public Health Rep* 1982;5:417–37.
- [22] Kiecolt-Glaser JK, Garner W, Speicher CE, Penn G, Glaser R. Psychosocial modifiers of immunocompetence in medical students. *Psychosom Med* 1984;46:7–14.
- [23] Rojas IG, Padgett DA, Sheridan JF, Marucha PT. Stress-induced susceptibility to bacterial infection during cutaneous wound healing. *Brain Behav Immun* 2002;16:74–84.
- [24] Ribble DO. The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. *Behav Ecol Sociobiol* 1991;29:161–6.
- [25] Xia XH, Millar JS. Genetic evidence for promiscuity in *Peromyscus leucopus*. *Behav Ecol Sociobiol* 1991;28:171–8.
- [26] DeVries AC. Interaction among social environment, the hypothalamic–pituitary–adrenal axis, and behavior. *Horm Behav* 2002;41:405–13.
- [27] Ribble DO. Lifetime reproductive success and its correlates in the monogamous rodent, *Peromyscus californicus*. *J Anim Ecol* 1992;61:457–68.
- [28] Modi W. Reproductive tactics among deer mice of the genus *Peromyscus*. *Can J Zool* 1984;62:2576–81.
- [29] Martin LB, Scheuerlein A, Wikelski M. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proc R Soc Lond, B Biol* 2003;270:153–8.
- [30] Demas GE. The energetics of immunity: a neuroendocrine link between energy balance and immune function. *Horm Behav* 2004;45:173–80.
- [31] Martin LB, Gilliam J, Han P, Lee KA, Wikelski M. Corticosterone suppresses immune function in temperate but not tropical house sparrows (*Passer domesticus*). *Gen Comp Endocrinol* 2005;140:126–35.
- [32] Castro WLR, Matt KS. Neuroendocrine correlates of separation stress in the Siberian dwarf hamster (*Phodopus sungorus*). *Physiol Behav* 1991;61:477–84.
- [33] Pyter LM, Neigh GN, Nelson RJ. Social environment modulates photoperiodic immune and reproductive responses in adult male white-footed mice (*Peromyscus leucopus*). *Am J Physiol, Reg* 2005;288:R891–6.
- [34] Remage-Healey L, Adkins-Regan E, Romero LM. Behavioral and adrenocortical responses to mate separation and reunion in the zebra finch. *Horm Behav* 2003;43:108–14.
- [35] Kiecolt-Glaser JK, Fisher L, Ogrocki P, Stout JC, Speicher CE, Glaser R. Marital quality, marital disruption, and immune function. *Psychosom Med* 1987;49:31–4.