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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.

Keywords: Deer mouse; Immune; Isolation; Social; Stress

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 (± 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 O_2 enriched-air and a patch of fur (approx. 90 mm^2) 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 CO_2 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 1400 h), 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 Kolmogrov–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.