Behavioural Brain Research 226 (2012) 606–612

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Environmental enrichment with nesting material accelerates wound healing in isolation-reared rats

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ARTICLE INFO

Article history:
Received 28 July 2011
Received in revised form
22 September 2011
Accepted 26 September 2011
Available online 13 October 2011

Keywords:
Environmental enrichment
Isolation rearing
Wound healing
Bedding
Nesting

ABSTRACT

Early enrichment (EE) programs provide a well-established approach to mitigate the deleterious effects of childhood adversity. To better understand the therapeutic features of EE, in the current study we compared the effect of two forms of nesting material on isolation reared (IR) rats. We found that both materials, absent of social and any other physical enrichment, significantly improved wound healing rates. The results suggest that this animal model may provide useful insights into the critical components of EE.

Early childhood psychosocial deprivation has well documented detrimental effects on behavior, health, and brain development in both humans [1–8] and, as we and others have shown, in isolation-reared (IR) animals [9–19]. The effects of psychosocial deprivation on wound healing are particularly striking, as we have previously noted [11], consistent with other studies showing a significant detrimental impact of environmental stress on wound healing [5,20–26].

Recently, we showed that environmental enrichment (EE) using Nestlets (Ancare, Bellmore, NY, USA, www.ancare.com), a commercially available cotton pulp material, can reverse the deleterious effects of psychosocial deprivation on IR rats [18]. EE also improves other aspects of physical and behavioral health as described elsewhere [27–30]. The effect of EE on wound healing in IR rats has not been examined with the exception of our earlier study [18]. Studies examining the effects of EE on IR rats have generally not well differentiated between EE and social rearing (with the experimental group often containing both physical and social EE), and the nature of the EE provided in those studies varied from simple nesting material to more complex objects such as toys and ladders [31–40]. However, some findings have shown a dissociable effect of EE containing (but not limited to) physical stimuli versus social enrichment without EE objects [37,41,42].

The importance of distinguishing differential health benefits of social from non-social enrichment, and among different forms of non-social enrichment provides the opportunity to make basic science research on EE more clinically relevant. This is because, as with many of the animal studies just reviewed, early enrichment

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doi:10.1016/j.bbr.2011.09.038

programs for high risk children often utilize multi-modal interventions involving a combination of sensory and social inputs [43–47], making it difficult to determine which component is responsible when there is an overall benefit [43–48].

As an initial attempt to identify critical factors involved in the benefit of EE in IR rats, in the current study we compared the beneficial effect of nesting material enrichment on wound healing previously observed with Nestlets [18], to enrichment with CareFRESH®, a wood pulp nesting material (International Absorbents, Inc., Ferndale, WA, USA, (www.absorptioncorp.com). A focus on subtle bedding differences was supported by prior studies that suggested nesting material is a particularly potent form of EE in rodents [30,49–52]. We chose CareFRESH bedding because of both its similarities to and differences from Nestlets. While both materials are supplements to the usual chipped hardwood bedding provided to the rats in our laboratory, CareFRESH originates from wood while Nestlets are derived from cotton. In addition, rats must shred Nestlets by using their mouths and forepaws, while CareFRESH is pre-shredded and requires less oral motor activity to create a nest of bedding. Results showed that both forms of EE effectively reversed the impaired wound healing for IR rats, suggesting that factors common to both forms of nesting material EE likely account for their therapeutic effect.

The animals were maintained in accordance with National Research Council guidelines [53] and the experimental protocols were approved by the Subcommittee on Research Animal Care, Massachusetts General Hospital. A total of 25 male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were obtained on post-natal day (PN) 17 with lactating dams.

On PN 20, rats were administered a third degree 20% total body surface area scald burn following the procedure described previously [11,18]. Briefly, rat pups were removed from the cage with their dam and anesthetized with ketamine (80 mg/kg intramuscular) and xylazine (12 mg/kg intramuscular). Hair surrounding the mid-dorsal surface was removed by an electric razor and the rats were then placed dorsal surface down, in a mold exposing the thoracic dorsal surface of the skin to preheated water (92 °C) for 8 s. Immediately afterwards, the rats were administered sterile saline (50 ml/kg intraperitoneal) while they recovered from anesthesia.

Animals were housed at the same institution as in our prior studies of wound healing [11,18], but in a different animal facility that utilized sterilized microisolator cages with forced ventilation, versus static (passive air exchange) microisolator cages in the prior studies. As in the prior studies, after burn administration, and while still under anesthesia, rats were randomly divided into four different housing conditions which all had standard rat bedding (chipped hardwood, see Fig. 1) but varied according to the presence or absence of social enrichment or nesting material enrichment as follows:

1) In the condition designed to model the absence of both social and nesting enrichment, isolation-reared (IR) rats were singly housed without nesting material ($n = 7$). See Fig. 1, panels 1a and 1b.
2) In the condition designed to model the presence of social enrichment and the absence of nesting or other physical enrichment, group-reared (GR) rats were housed with littermates in two groups of three without nesting material ($n = 6$). See Fig. 1, panels 2a and 2b.
3) In the first condition designed to model the absence of social enrichment and presence of nesting enrichment, Nestlet-enriched (NE) rats ($n = 6$) were singly housed in standard cages with new Nestlets provided twice weekly at the time of cage changing. Nestlets are chemically inert, odorless, non-ingestible one-by-one inch squares of sterilized pulp virgin cotton fiber. Rats chew and tear the Nestlets into pieces that they pile together in which to rest. See Fig. 1, panels 3a and 3b.
4) In the second condition designed to model the absence of social enrichment and the presence of nesting enrichment, CareFRESH-enriched (CE) rats ($n = 6$) were singly housed in standard cages with two ounces of CareFRESH provided twice weekly at the time of cage changing. CareFRESH beddingis a soft, greyish wood pulp that, when placed in the center of cage at the time of cage changing, forms a loose, low density pile. The rats burrow and gnaw on the strands of the pulp to gradually form a resting area. See Fig. 1, panels 4a and 4b.

The pictures were quantitatively analyzed as in our previous studies [11,18]. Briefly, every seven days post burn injury (week 1, 2, 3, 4 post burn injury at PN 27, 34, 41, 48, respectively), while under isoflourane anesthesia, the dorsum of each rat was photographed such that the area of unhealed wound tissue could be compared to the entire dorsal surface of the rat, excluding regions above the neck and below the hindquarters. Each picture was taken from approximately the same height. The photographs were quantitatively analyzed as in our previous studies [11,18]: using Photoshop software (http://www.photoshop.com), the number of pixels in the unhealed area was normalized to the area comprising the total dorsum of each rat. The total dorsum area was determined by multiplying the length of the dorsum by the greatest width of the body. Normalization to the dorsal surface of the animal accounted for differences in wound size measurement due to the varying sizes between animals. Comparable percent healing scores were obtained independently by three different research assistants, including two authors (AV, JF). The Student's *t*-test for unpaired data with assumed unequal variance was used to statistically compare the difference in the area of unhealed wound tissue between rats in the four conditions listed above (IR, GR, NE, CE).

The results, shown in Figs. 2 and 3 and Table 1, indicated that as in our previous studies [11,12,18], IR rats without EE did not heal effectively even four weeks post burn injury (PN 48), with significantly larger residual open wound tissue compared to all other conditions at the end of the study (row 4, column 2, Fig. 2; solid grey line week 4, Fig. 3; Table 1A, column 9, rows 1–3). In contrast and consistent with the prior findings, GR rats healed with well apposed wound margins at four weeks post burn injury (row 4, column 1, Fig. 2) and with significantly less open wound tissue compared to all other conditions at week 4 (solid black line, week 4, Fig. 3; Table 1A, column 9, rows 1, 4 and 5). Furthermore, as in our prior study, the singly housed NE rats also developed well healed wounds by four weeks post burn injury (row 4, column 3, Fig. 2) with significantly less open wound tissue compared to IR rats (dashed black line, week 4, Fig. 3; Table 1A, column 9, row 2). Finally, at week 4, the singly housed CE rats showed comparable healing to the NE rats, with well apposed margins evident by week 4 post burn injury (row 4, column 4, Fig. 2), and with significantly less open wound tissue compared to IR rats (compare dotted grey line to solid grey line, week 4, Fig. 3; Table 1A, column 9, row 3) that was not different in open tissue compared to the NE rats by week 4 (compare dotted grey line to dashed black lines, Fig. 3 and Table 1A column 9, row 6).

Of note, although the amount of open wound tissue of NE and CE rats was much closer to the GR rats than the IR rats, their residual open area at week 4 was statistically greater than GR rats (compare dotted grey line and dashed black lines to solid black line, week 4, Fig. 3; Table 1A, column 9, rows 4 and 5). This contrasts with our prior study, wherein we saw that by week 4 the healing of NE rats was not statistically different than the healing of GR rats. Thus, in the current study, although nesting material and social enrichment each resulted in substantially greater wound closure compared to the IR rats reared without either enrichment (Fig. 3, compare all