



Social isolation increases cell proliferation in male and cell survival in female California mice (*Peromyscus californicus*)



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HIGHLIGHTS

- *Peromyscus californicus* mice were housed in isolation for 4 or 24 days.
- Male mice isolated for 4 days increased cell proliferation in the hippocampus.
- Female mice isolated for 24 days increased cell survival in the hippocampus.
- Results indicate sex and species specific cellular patterns following isolation.

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ABSTRACT

Social environment has direct effects on an animal's behavior, physiology and neurobiology. In particular, adult neurogenesis is notably affected by a variety of social manipulations, including social isolation. We hypothesized that social isolation should have particularly acute effects on neurogenesis in a highly social (monogamous and bi-parental) species such as *Peromyscus californicus*, the California mouse. Adult male and female *P. californicus* mice were housed in isolation or in same-sex pairs for 4 or 24 days. At the end of each period, either cell proliferation or cell survival was quantified with BrdU label and neuronal markers (either TuJ1 or NeuN). After 4 days, isolated males had greater cellular proliferation in the dentate gyrus of the hippocampus (DG) than pair housed males. After 24 days, isolate females demonstrated greater cell survival in the DG than paired females. Males demonstrated a similar, but non-significant pattern. No differences in cellular proliferation or cell survival were found in the subventricular zone (SVZ), or medial amygdala (MeA). These results add to the evidence which demonstrates that neurogenic responses to environmental conditions are not identical across species. These data may be critical in understanding the functional significance of neurogenesis as it relates to the interactions between social systems, social environment and the display of social behaviors.

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1. Introduction

Social isolation can have profound effects on an animal's physiology and behavior. Among rodent species isolation can result in changes in social behavior, stress reactivity and neuroendocrine function. The nature and degree of these effects are not necessarily uniform across species and vary as a function of the social organization of the species [1–3]. Adult neurogenesis in established proliferative zones (dentate gyrus of the hippocampus and subventricular zone of the lateral ventricle) is also affected by various manipulations of the social environment [4–7]. Whereas, various forms of environmental or social enrichment tend to increase neurogenesis, environmental or social stressors tend

to decrease neurogenesis [8–10]. However, several exceptions and variants on this pattern of results have been demonstrated. More accurately, the precise relationship between a stressor and neurogenesis relies on a number of factors including the type of stress, its duration and developmental timing [3,11–13]. Furthermore, following the same stressor, patterns of neurogenesis are region specific [7,13]. Neurogenesis patterns also demonstrate sex and species differences in response to the same stressor [14,15]. Consequently, much remains to be understood regarding the functional significance of these patterns and their potential relationship to social behaviors [16].

There are several studies which demonstrate a functional significance of neurogenesis within either the DG or SVZ, associated with learning, the efficacy of antidepressants and other behavioral outcomes [17–19]. In many of these cases, it is an increase in neurogenesis that enhances or facilitates functionality. However, patterns of neurogenesis following isolation are less consistent, making predictions associated

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with functionality and behavior more difficult. Social isolation can result in increases, [20,21] decreases [3] or no change [14] in measures of cell proliferation or survival; patterns which vary with brain region (DG or SVZ), sex, species and isolation paradigm. Other stressors show similar sex and species variance in their effects on neurogenesis. Acute restraint stress decreases cell proliferation in rats, but increases cell proliferation in mice [22]. Male rats show decreases in cell survival in the DG following chronic electric shock, whereas individually housed females show increases [14]. These results make straightforward predictions of a stressor decreasing neurogenesis tenuous amidst clear, but fragmentary evidence across species and stress paradigms.

Beyond the functional implications there are also proximate differences in the mechanisms of neurogenesis that vary with sex and species. New neurons differentiate faster and are more functionally significant in the hippocampus of rats than mice [15]. Neurogenesis rates vary relative to age and as it relates to life history stage of a given species [11]. Therefore, cross species comparisons of neurogenesis are confounded not only by differences in life history, but by the specific mechanisms of the neurogenic process.

A comprehensive understanding of the role of neurogenesis as it relates to social behavior (or in response to social environment) is only beginning to emerge. Comparisons across species with various life histories will help elucidate this understanding. For example, manipulations of the social environment are known to affect neurogenesis in a monogamous bi-parental rodent, the prairie vole, *Microtus ochrogaster*. Sexual experience, social isolation [20], and the display of parental behavior [23] all differentially affect rates of neurogenesis in this species. There are also apparent sex differences in neurogenesis patterns in relation to social environment likely the result of estrogenic function and associated neuroendocrine responses [24,25]. Isolation is also known to have a number of anxiety-related behavioral effects in the prairie vole [26]. However, if neurogenesis contributes these behavioral deficits has not been determined.

Neurogenesis is affected by behavioral stimuli (paternal behavior) and environmental manipulations in the California mouse (*Peromyscus californicus*), as well as other rodent species [4,16,23,25]. Although both the California mouse and prairie vole are monogamous and bi-parental the similarity in their behavioral repertoires are the result of convergent evolutionary processes. Potential similarities in the patterns of neurogenesis may not necessarily be the result of homologous neurobiological processes, but could indicate similar mechanisms of neurogenesis in relation to mating systems. In the present study we quantified rates of neurogenesis in the California mouse following 4 or 24 days of isolation or paired (same-sex) housing. By quantifying cell proliferation, cell survival and neurogenesis in this monogamous, bi-parental rodent [27,28] we aim to provide further comparative evidence regarding species specific patterns in response to social environment. Manipulations of the social environment may have particularly acute effects in this highly social species. Changes in cell proliferation, cell survival and neurogenesis rates may underlie behavioral changes associated with social isolation or other social stimuli.

2. Methods

2.1. Subjects and housing conditions

Subjects were laboratory-bred male and female California mice (*P. californicus*) derived from individuals purchased from the *Peromyscus* Stock Center, University of South Carolina, Columbia, SC. Animals were maintained on a 16:8 light: dark cycle and allowed food (Harlan Teklad Rodent Diet 8604) and water ad libitum. Subjects were weaned at 24 days of age and placed in same-sex pairs with siblings or strangers of similar age. Between 45 and 66 days of age animals were removed from these pairs and placed into one of the following conditions: isolation (singly housed), pair housed (housed with a sibling or stranger of the same sex and age). Cages were polypropylene (opaque) 11"

long × 8.5" wide × 6" deep with stainless steel wire lid with bedding (shredded aspen shavings). Conditions were maintained for 4 or 24 days. All husbandry and experimental procedures were approved by an IACUC committee, protocol number 2012–17.

2.2. BrdU injections

BrdU (5-bromo-2'-deoxyuridine) is an established marker of cellular proliferation incorporated in the DNA while cells are in the S-phase of the cell cycle. BrdU was prepared the day of injection by dissolving BrdU (Sigma–Aldrich, #B5002) in a 0.9% saline solution with NaOH (0.007%) to a concentration of 20 mg of BrdU/ml of solution and then filtered. Subjects received two intraperitoneal injections 6 h apart on the third day of the housing conditions. Timing of injections relative to the circadian cycle was similar across all subjects: first injection 11–12 h and second 17–18 h. The third day of housing was chosen for injections to allow for approximately 24 h for BrdU label to occur prior to euthanizing the first group of subjects (cell proliferation) and to allow for 21 days for BrdU label to ensure an accurate measure of cell survival within in the second group. Although the precise time course for cell survivability is not known in this species, 3 weeks is representative of the time course demonstrated in both rats (*Rattus*) and mice (*Mus*) [3]. Subjects were injected with 0.5 ml of solution/100 g of body weight (200 mg/kg). This timing of the injections and dosage was previously found to be effective in labeling cell proliferation and survival in cells in the prairie vole [20,23,32].

2.3. Fixation, sectioning and immunocytochemistry for BrdU, BrdU/TuJ1 and BrdU/NeuN

Animals were euthanized with an overdose ketamine/xylazine and rapidly perfused with a 0.9% saline followed by a 4% paraformaldehyde. This was approximately 24 h (cell proliferation) or 21 days (cell survival) after the second BrdU injection. All animals were euthanized between 10 h–14 h. Brains were extracted and post-fixed for 4 h in 4% paraformaldehyde. Brain tissue was coronally sectioned on a freezing microtome at 40 µm. Sections were collected beginning at the most rostral level of the anterior commissure through the entire hippocampus and the most rostral portions of the cerebellum (approximately 90–100 sections per animal). Sections were placed in cryoprotectant and stored at –20 °C until processing for immunocytochemistry.

Alternate sections (every sixth) were processed for either BrdU fluorescent immunocytochemistry (single label) or BrdU with either TuJ1 or NeuN (double label fluorescent immunocytochemistry to discern neuronal phenotype). For all immunocytochemical reactions, tissue from a near equal number of subjects from each condition (males and females, isolate and paired) was included. In the cases where subject numbers were not identical, at least two subjects from every condition were included in every run of immunocytochemistry. Sections were rinsed in KPBS, placed in a NaBH₄ solution for 20 min, rinsed in KPBS, denatured in for 1.5 h. in a 2 M HCl solution at 37 °C and rinsed again in KPBS. Sections were then incubated in a rat anti-BrdU monoclonal primary antibody (Accurate Chemical, #OBT0030) at a concentration of 1:200 for 1 h. at room temperature and overnight at 4 °C. Following KPBS rinses sections were placed in the secondary antibody (AlexaFluoro 546, Invitrogen #A-11,081) at concentration of 1:200 for 1.5 h.

Sample brain tissue from animals not injected with BrdU was compared with sample brain tissue from experimental animals (injected with BrdU) prior to collection of any experimental data. This tissue came from animals that were not subjects and served as a negative control for non-specific BrdU antibody reactivity. Liver tissue was collected from each subject and the presence of BrdU-ir cells in liver tissue was confirmed for each run of immunocytochemistry and if necessary used to verify that low levels or the absence of BrdU-ir in brain tissue was attributable to housing condition (as opposed to variability in BrdU injections or immunocytochemistry). Staining within the liver

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