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Research report

Seasonal and sex differences in the hippocampus of a wild rodent

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HIGHLIGHTS

- Few studies have examined both seasonal and sex differences in hippocampus size.
- We examined seasonal and sex differences in hippocampus size of Richardson's ground squirrel.
- Males have the largest hippocampus volumes in non-breeding season.
- Dentate gyrus, CA1 and CA3 varied between sexes and seasons.

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ABSTRACT

Studies across and within species suggest that hippocampus size is sexually dimorphic in polygamous species, but not in monogamous species. Although hippocampal volume varies with sex, season and mating system, few studies have simultaneously tested for sex and seasonal differences. Here, we test for sex and seasonal differences in the hippocampal volume of wild Richardson's ground squirrels (*Urocitellus richardsonii*), a polygamous species that lives in matrilineal, kin-based social groups and has profound sex differences in behavior. Based on the behavior and ecology of this species, we predicted that males would have a significantly larger hippocampus than females and that the hippocampus would be largest in males during the breeding season. Analyses of both absolute and relative volumes of the hippocampus yielded a significant difference between the sexes and seasons as well as an interaction between the two such that non-breeding males have significantly larger hippocampal volumes than breeding males or females from either season. Dentate gyrus, CA1 and CA3 subfield volumes were generally larger in the non-breeding season and in males, but no significant interaction effects were detected. This sex and seasonal variation in hippocampal volume is likely the result of their social organization and male-only food caching behavior during the non-breeding season. The demonstration of a sex and seasonal variation in hippocampal volume suggests that Richardson's ground squirrel may be a useful model for understanding hippocampal plasticity within a natural context.

1. Introduction

Numerous studies in rodents have demonstrated that spatial ability is associated with hippocampal function [1,2] and with mating strategy and/or range size [3,4]. In polygamous species, the hippocampus is a sexually dimorphic structure; it is significantly larger in males than in females [5–8]. Many of these same species also exhibit a male advantage for spatial tasks including: meadow voles (*Microtus pennsylvanicus* [9]), deer mice (*Peromyscus maniculatus* [10]), C57BL/6J mice (*Mus musculus* [11]), Wistar rats (*Rattus norvegicus* [12]) and humans (*Homo sapiens* [13]). Thus, sexual dimorphism in the volume of the hippocampus appears to be related to spatial ability (for review see [14]) in polygamous species.

One of the putative reasons for this sexual dimorphism in hippocampal volume and spatial ability is that in polygamous species, males have much larger home ranges and travel greater distances than females [3,4,6,7,15–17]. This relationship is corroborated by comparisons within other mating systems. For example, in avian brood parasites [18,19] and polygynandric fish [15], females range over much larger areas than males and there is a corresponding female-bias in the volume of the hippocampus (or its homolog in fish, the dorsolateral telencephalon). Furthermore, in monogamous species in which there is no sex difference in home range size, there is no difference in hippocampal volume [6]. Thus, there appears to be an interplay among mating system, space use and hippocampal volume, such that where a sex difference occurs in home ranges, which generally accompanies polygamous mating systems, there is a corresponding sex difference in both hippocampal volumes and spatial abilities.

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Hippocampal volume is not, however, static. In several mammals and birds, hippocampal volume varies seasonally [20–27] and in response to changes in endogenous concentrations of steroid hormones [5,26,28,29]. Seasonal variation is largely tied to seasonal differences in behavior; the hippocampus is larger in the season that places more spatial demands on the animal (but see [30]). For example, in brood parasitic cowbirds (*Molothrus* spp.), hippocampal volume is largest during the breeding season when host nests have to be located [20]. In mammals, shrews and voles exhibit marked seasonal changes in hippocampus size (smaller in fall/winter), which appears to coincide with seasonal variation in home range size [27]. Studies of food caching species, however, have failed to detect consistent seasonal changes in hippocampal volume, despite seasonal differences in food caching [21–24,30,31]. Despite the mixed evidence for seasonal changes in hippocampal volume of wild vertebrates [30–32], hippocampal size does vary with natural fluctuations in endogenous steroid hormone levels [5,26,29] and therefore seasonal changes could occur in many species, but has simply not been investigated in detail.

Overall, these inter- and intraspecific studies suggest two generalities regarding sex and seasonal differences in hippocampal volume. First, sex differences in hippocampal volume should occur in any species in which there is a significant difference in home range size. Second, in species in which there are significant seasonal differences in spatially dependent behaviors, there is often a corresponding change in hippocampal volume. Despite the potential for an interaction between seasonal variation and sex differences, these two hypotheses have only been tested simultaneously in two studies. Clayton et al. [20] examined the effects of sex and season on hippocampal volume in two cowbird species (*Molothrus bonariensis* and *M. rufoaxillaris*). In both species, the hippocampus was significantly larger in breeding than in non-breeding seasons and, in one species, there was a significant sex difference in relative hippocampal volume. No significant season by sex interaction was detected, but the sex difference was smaller in the non-breeding than in the breeding season in one of the two species. Lavenex et al. [31] also tested for seasonal and sex differences in hippocampal volume, as well as other neuroanatomical measurements of the hippocampus, in eastern gray squirrels (*Sciurus carolinensis*). They found significant seasonal and sex differences in absolute volume of the entire hippocampus, dentate gyrus and CA1 and CA3 subfields, but relative to brain volume, only CA1 was significantly different between the sexes and there were no significant interaction effects. Given that these two studies addressed seasonal and sex differences in disparate species and yielded divergent results, further studies are required to determine the extent to which a combination of seasonal variation and sex differences affect hippocampal volume.

An adequate test of the interaction between sex and season should focus on a species in which there are marked seasonal and sex differences in spatially demanding behaviors. In addition, a wild population should be examined because of the potential for captivity to have confounding effects on hippocampal volume [22,26,33–38]. Here, we provide such a test of both sex and seasonal differences in hippocampal volume in a wild population of Richardson's ground squirrels (*Urocitellus richardsonii* [39]). The Richardson's ground squirrel is a prime candidate for testing the combined effects of sex and season because of profound sex differences and seasonal variation in their behavior and ecology [40]. Male squirrels differ from females in: body mass, body composition, growth pattern, survivorship, timing of entrance to and emergence from hibernation, amount of time spent in torpor, dispersal distance, food caching, relative brain volume and home range size [40–45]. In fact, the home range of a male is 5–18 times greater than that of females [43]. The males cover this massive area (up to $8618\,m^2 \pm 1412\,m^2$ [43]) with borders that fluctuate daily [42], in order to find and mate with females (home ranges = 428–1724 m^2), which have only a 24 h estrous period [43,46]. This sex difference in range size declines significantly following the breeding season [47]. Thus, Richardson's ground squirrels exhibit substantial sex differences in space use that vary seasonally.

Richardson's ground squirrel behavior also varies considerably throughout the year. Following the breeding season, both sexes begin to prepare for hibernation by spending most of their time feeding and preparing a chamber, known as a hibernaculum, for winter. In males, part of this preparation includes making a larder of stored seeds within their sleeping chamber [40,48,49]. Depending on age and sex, Richardson's ground squirrels spend 4–8 months in hibernation [40]; thus, a significant part of the year is spent in torpor. The hippocampus plays a role in the induction of hibernation [50–52] and undergoes significant changes in its anatomy and physiology during hibernation and arousal in the spring. These changes have been characterized in other species of ground squirrels and include: a large decrease in pyramidal cell soma size [53–55], decreases in dendritic branching and spine density of CA1 and CA3 cells [53–55], fewer mossy fiber terminals [56,57] and up to a 65% loss of synapses [58,59]. Upon arousal from hibernation, there is a rapid increase in cell soma size, dendritic branching and spine density within several hours [53,54,57,58] and this appears to parallel a recovery of function based on behavioral tests [60]. However, none of these studies examined sex as a variable. Thus, there are both behavioral and anatomical data suggesting that hippocampal volume might undergo seasonal changes in Richardson's ground squirrels, but the extent to which these changes vary between the sexes is unknown.

Based on the ecology and behavior of Richardson's ground squirrel, and studies of other species, we predicted that both seasonal and sex differences in hippocampal volume would be present. More specifically, males should have larger hippocampal volumes than females and hippocampal volume should be largest during breeding season because home range sizes are larger. If home range size exerts the strongest influence on hippocampal volume, as predicted by the studies of other polygamous species [3,4,6–8,15–17], then there should also be an interaction effect whereby the hippocampus is largest in males during the breeding season because they occupy much larger home ranges than the females, the males appear to track where estrous females are within the colony and the home ranges are larger in the breeding season than at other times of the year [40,43]. Here, we specifically test these predictions in wild caught Richardson's ground squirrels.

2. Materials and methods

2.1. Animals

All of the procedures outlined below adhered to the Canadian Council for Animal Care guidelines and were approved by the University of Lethbridge Animal Welfare Committee. Collection and research permits were issued by the Alberta Department of Sustainable Resource Development.

50 Richardson's ground squirrels were caught on campus at the University of Lethbridge and several private properties near Lethbridge, Alberta, Canada. We divided our trapping activities into two seasons: breeding season (February–April) and non-breeding season (July–September). These two times approximate the time of emergence from hibernation and mating season (February–April) and the period just prior to immergence into hibernation (July–September; [40]). All of the squirrels were caught in Tomahawk (Model #102, 41 cm × 13 cm × 13 cm, Tomahawk Live Traps, Tomahawk, WI) or Safeguard (Model # 50450, 46 cm × 13 cm × 13 cm, Safeguard Products, New Holland, PA) single door live traps placed at burrows and baited with black oil sunflower seeds and/or spinach and lettuce. Adults in our non-breeding season sample were distinguished from juveniles of that year by larger body mass [40], tooth color and, in the case of males, the presence of scars and other physical damage arising from breeding season (e.g., missing digits). One or more observers constantly monitored the traps from a distance of 30–50 m with binoculars. The squirrels were removed as soon as possible once trapped, placed into a zippered, cone-shaped bag, weighed to the nearest 5 g with a spring scale