

**Effects of stress on decision making in the  
Merriam's kangaroo rat (Dipodomys merriami)**

by

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B.A. (University of Virginia) 1995

M.A. (University of California, Berkeley) 1998

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Psychology

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, BERKELEY

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Fall 2001



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University of California, Berkeley

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## **Abstract**

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Doctor of Philosophy in Psychology

University of California at Berkeley

Professor Lucia Jacobs, Chair

Kangaroo rats are granivorous animals that live in semi-arid zones where seed production is unpredictable. By caching food when it is available, kangaroo rats can create a predictable supply of food despite the variability in production. Merriam's kangaroo rats are specialized for scatter hoarding (caching seeds in multiple, dispersed locations, each used only once), but they also use larder hoarding (caching all seeds in a single location that is reused). Cache decisions require an animal to take into account many variables, such as the availability of food, the risk of pilferage and the risk of predation. These variables must then be used to determine the optimal proportion of scatter and larder hoarding, the size of each cache, and the location of each cache. Thus, caching in Merriam's kangaroo rats is a cognitive and economic decision that can be used to investigate decision-making processes.

Seven laboratory experiments with wild-caught Merriam's kangaroo rats demonstrated the flexibility of cache decisions. Although this species is characterized by its specialization for scatter hoarding, there is great within- and between-individual variation in the actual proportion of scatter and larder hoarding, depending on the external social conditions and the internal state of the animal at the time of caching. Experimental conditions that affected cache strategy included pilferage by a familiar conspecific individual, pilferage by a dominant heterospecific individual, and food deprivation.

Food competition from pilferage may be stressful to the animals. Food deprivation is a known physiological stressor that increases circulating levels of glucocorticoid hormones. Thus, a glucocorticoid-based proximate model for cache decision-making was hypothesized and tested. Blood samples from before and after caching, and in response to classical stressors such as food deprivation and physical restraint, were collected and analyzed through radioimmunoassay. Results from these experiments are insufficient to support a glucocorticoid-based mechanism for cache decisions. This may be due either to a sampling confound, or to the ecology of this desert-living species.

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Professor Lucia F. Jacobs

Dissertation Committee Chair

To Brent Stansfield

He is the champ.

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## **Acknowledgements**

I would foremost like to thank Brent Stansfield for his love, support, and practical help throughout the period of this dissertation work. He is there for me, always.

My family provided me with a model for intellectual achievement. My spirit is their spirit. With their high-octane formula of enthusiasm, curiosity, “stick-to-it-iveness”, and cookies, I was able to keep the engine running, even when the hills were long and tall.

I would like to thank my friends for their interest, intelligence, and for always being there when I was ready to cut loose from the dissertation strings.

I would like to thank my advisor, Lucy Jacobs for her enthusiasm for research and for allowing me to follow my own research heart. Her own discoveries in the field of cognitive ethology were the starting point for all of my kangaroo rat experiments; she showed me that interesting cognitive research can be done with complex, natural species.

My committee, including Lucy Jacobs, Steve Glickman and Eileen Lacey, was supportive and provided much needed constructive criticism, especially in the inchoate stages of my proposal.

**LAB** I would like to thank all of the members of the Jacobs lab, especially Cynthia Barkley, Sasha Gibbs, Melissa Adams and Kate Onstott, for their social, moral and intellectual support. The occasional cup of coffee or water cooler conversation made the drudgery of research a pleasant burden to bear.

**RESEARCH** I am indebted to all of the research assistants that cheerfully helped me with this research, no matter how tedious, dusty, and back-breaking the work could be. Melissa Adams worked expertly on the place preference experiments; Candace Basich worked on animal care, food deprivation experiments, and individual cache preference experiments; Jay Nagtalon worked on many small, multiple-subject experiments and was generally very helpful in the final stages of this research. Erin Kishi was also helpful with animal care and research maintenance. The members of the Zucker and Breedlove labs were always approachable and friendly; I am especially indebted to Bradley Cooke and Chris Tuthill. The individuals in these labs helped me to better understand research procedures and lent me equipment when I was without, even the expensive kind.

**ANIMAL CARE STAFF** I would like to give a special thanks to Nina Hahn, who was very approachable and helpful throughout this research, even going out of her way to help troubleshoot animal care and blood collection problems. Silvario Cuaresma helped design and teach the blood collection method. Jose Castillo was very friendly, approachable, and helpful with all matters of technique. Bennie Thomas and Joan

Wallace deserve a special commendation for always being friendly and respectful, even when there were problems. Of course, I would like to thank all of the care staff that took care of the kangaroo rats on a daily basis, and made the NAF a generally nice place to work. Thanks also to John Parker, Keith Jenné and Lindsey Jennings. Al Rollins, the security guard at the NAF, is the greatest. Not only was he there at all hours, he was a model of friendliness, openness, warmth and citizenship. I will really miss him.

**PSYCHOLOGY DEPARTMENT**            Thanks very much to all of the people who work in the Psychology office and help make research possible, despite great bureaucratic impediments. Special thanks to Johanna Cates for help with ordering and Judy Fong for help with grants. Thanks also to José Lopez and Ted Claire of the machine shop, for their expert arena design and construction. Thanks to Ted Crum and Eric Eichorn for solving all of our computer problems with the greatest of ease.

**ANIMAL PROCUREMENT**            Al Muth at the UC Riverside Research Station in Palm Desert, CA helped to arrange for my trapping trip in January of 2000 and was very accommodating and helpful during that trip. Martin Daly provided his traps during this trip. Shana Dodd generously trapped animals and helped me to trap additional animals in Palm Springs, CA in April of 2000.

# Chapter I

## **A General Introduction to Stress, Decision Making, and the Species**

### **I.1. The effects of stress on decision making and memory**

Decision making is a complex process whereby animals must integrate information about conditions in the environment with information about their internal state and make predictions about future conditions and needs. The allocation of resources is a particular category of economic decision-making that involves everything from decisions about the number of offspring to sire to the investment of income in the stock market. Animals must have enough resources to allow for the reproductive success of themselves and their offspring, balancing risk-prone and risk-avoidant behaviors such that benefits can be enjoyed without an undue effect from costs (Krebs & Davies, 1994). Despite the pervasiveness and importance of these decisions, little is known about the link between emotions and these decisions. Much research reports an inverted-U shaped curve of arousal and memory (for reviews see Cahill & McGaugh, 1996; Joseph, 1999), and of arousal and performance (e.g. Yerkes & Dodson, 1908; Parfitt et al., 1995; Van Londen et al., 1998), but the interrelationship between emotion, decision making and memory is poorly understood.

Human and non-human research into decision making has been almost entirely focused on cognitive models of optimization; when emotion is incorporated into these models, it is without the benefit of a proximate or physiological model. A few experiments with domesticated rats and mice have concurrently investigated the effects of arousal-mediating substances on decision making and memory, but the results are mixed (laboratory rats: Gargiulo et al., 1996; de Souza Silva et al., 1993; Viana et al., 1994; Watson, et al., 1994; Conrad et al., 1997; Tomaz et al., 1993; laboratory mice: Belotti et al., 1998). In studies with laboratory rats, some find that arousal-mediating drugs alter risk taking without effects on memory (Herman et al., 1991), some find that decreasing anxiety also decreases memory (de Souza Silva et al., 1993; Viana et al., 1994) and others find that drugs that increase risk taking effect memory non-selectively by increasing exploration and locomotion (Watson et al., 1994; Conrad et al., 1997).

In my previous research with Merriam's kangaroo rats I found that in the laboratory, competition for food affects both the emotional state and the food-storing strategy of subjects (Preston & Jacobs, in press). Based on these data, I hypothesized a non-linear relationship between arousal and food storing with metabolically/cognitively conservative strategies occurring at low and high levels of arousal and a metabolically/cognitively demanding strategy occurring at intermediate levels (Preston, 1998).

In order to understand the behavior of an organism, it is crucial to place it in the natural social and physical context. A rich knowledge of behavior and its underlying

physiology allows us to predict behavior not just in the species of interest, but in all species that share the same environmental and evolutionary constraints.

The behavioral and physiological research reported here can contribute to our understanding of behavior by augmenting current research with information regarding mechanism, adaptation and phylogenetic continuity. Merriam's kangaroo rats, due to their specialization for hoarding food and the relative ease of studying their behavior in the laboratory, offer an ideal system for integrating these three topics in a natural and complex setting.

## **I.2. Kangaroo rat ecology**

Merriam's kangaroo rats (*Dipodomys merriami*) are rodents in the family Heteromyidae. Heteromyids are characterized by a large, fur-lined cheek pouch that opens next to the mouth, extends back along the shoulders, and can be used to transport food (Myers, 1999). Most heteromyids are granivorous, polygynous, nocturnal animals that stay in burrows during the day and forage during the night (Zeng & Brown, 1987). Kangaroo rats (genus *Dipodomys*), are widely distributed across arid zones of North America (Daly et al., 1992b).

Merriam's kangaroo rats are well adapted to their environment with stable populations across seasons great variations in weather, temperature, and food supply and a high juvenile and adult survival rate despite having relatively small litters, and few litters per year (Zeng & Brown, 1987).

They are temporally opportunistic breeders that rely on the “green grass factor”, breeding after heavy rainfalls when vegetation production surges. This fecundity predicts later seed availability and is thus a good proximate cue for reproduction. This temporal reproductive variability is shared by many species in the genus Dipodomys; however, it is more extreme in the Merriam’s kangaroo rat than in five coexisting species of desert rodents in eastern California (Kenagy & Bartholomew, 1985).

Merriam’s kangaroo rats are relatively altricial compared to other Dipodomys species. There is a high frequency of adult dispersal and a high success in dispersing; these traits also exist in other Dipodomys species, but are again more extreme in Merriam’s (Zeng & Brown, 1987).

Merriam’s kangaroo rats have less elaborate day burrows than many of the other Dipodomys species, they frequently switch between day burrows, abandon them, and rarely collect significant amounts of food in them (Daly et al., 1992b). Generally, their burrows are found at the periphery of the home range, which distinguishes them from central-place foraging rodents that have burrows in the center of their home range (Daly et al., 1992b).

Merriam’s kangaroo rats are distinguished by their solitary and dispersed social system with overlapping territories. The territories of Merriam’s males generally overlap with those of other males and females, but female territories do not overlap (Behrends et al., 1986; Jones, 1989). Because of the overlap, neighbors encounter each other regularly, creating a “dear enemy” situation where neighboring individuals recognize each other

and are more tolerant and less aggressive towards each other than towards strangers (Randall, 1991; Temeles, 1994; Yoerg, 1999). This situation may facilitate mating since females are familiar with the males in overlapping territories, increasing the likelihood of mating (Randall, 1991).

Because Merriam's kangaroo rats live in overlapping territories, their foraging and cache decisions must take the presence of other individuals and other individual's caches into account. For example, social factors such as the presence of a competitor while caching, the distance of the cache to the burrow of a competitor, or the distance of a cache to the cache of a competitor are likely to be important for the survivability of the caches. These social factors have been researched in the caching literature of diurnal animals such as birds and squirrels (see the reviews in Chapters II and III), but little has been done with nocturnal cachers such as the Merriam's kangaroo rat. The effect of conspecific competition on caching was investigated in two experiments, described in Chapter III.

In addition to the intra-specific competition introduced by the overlapping territories, there is additional inter-specific competition from the fact that Merriam's kangaroo rats have large dispersal distances, and large home ranges with temporary day burrows. Merriam's kangaroo rats coexist with many other species in the same habitat, including many other granivorous rodents. Merriam's kangaroo rats have been found on the same sites as many other kangaroo rat species (e.g. Chisel-toothed kangaroo rat (Dipodomys microps), bannertailed kangaroo rat (Dipodomys spectabilis), Ord's

kangaroo rat (Dipodomys ordii), many pocket mouse species (e.g. pocket mouse (Perognathus parvus), Arizona pocket mouse (Perognathus amplus), little pocket mouse (Perognathis longimembris)), as well as deer mice (Peromyscus maniculatus), dark kangaroo mice (Microdipodops megacephalus), and long-tailed pocket mice (Chaetodipus formosus) (Brown, 1988; Jenkins & Breck, 1998).

These coexisting and competing species are referred to collectively as “desert rodent communities” and these communities are considered ideal models for interspecific interaction. Research is directed at determining the extent to which these species compete for resources, if this competition has led to the partitioning of food types or microhabitats (e.g. Brown, 1988; Jenkins & Ascanio, 1993; Price & Brown, 1983). Although not all of these species prefer the same seed species as Merriam’s kangaroo rat (e.g. *D. microps* is specialized for eating seeds with hard shells that *D. merriami* cannot eat), many of these species do eat the same seeds and there is evidence that some species can and will eat unpreferred foods when there is a shortage (Jenkins & Ascanio, 1993). The large home ranges of Merriam’s kangaroo rats make them especially vulnerable to competition from these sympatric species. Thus, it is also valuable to look at the effects of interspecific social variables on the cache strategy of Merriam’s kangaroo rats. As far as I know, there are currently no systematic studies on the direct effects of a sympatric, heterospecific animal on the caching of a kangaroo rat, although there is correlative field data on resource partitioning suggesting that Merriam’s kangaroo rats take the presence of these species into account when foraging and caching (e.g. Brown, 1988; Jenkins & Ascanio,

1993; Jenkins & Breck, 1998; Jenkins & Peters, 1992; Jenkins et al. 1995). The effect of heterospecific competition on caching was investigated in two experiments, discussed in Chapter II.

### **I.3. Foraging and cache behavior**

The foraging behavior of Merriam's kangaroo rats is specialized for survival in desert conditions. In semi-arid zones, the food distribution varies greatly in time and space (more so for greens than seeds; Bradley and Mauer, 1971). To ensure a constant food supply despite great variations in environmental conditions, many animals store food (Vander Wall, 1990). Their fur-lined, external cheek pouches can be used to transport seeds to temporary storage locations, called caches. There are two primary modes of caching: scatter hoarding and larder hoarding (Vander Wall, 1990). **Scatter hoarding** is dispersing food in multiple small caches (Morris, 1962), usually using each cache site only once (Vander Wall, 1990). **Larder hoarding** is keeping all food in a single site that is reused, like a burrow (Vander Wall, 1990). While animals are often characterized by their use of one or the other, the two are best thought of as ends on a continuum, since some animals use combinations of the two, and the density of scatter hoards varies, precluding the strict labeling of some distributions as one or the other (Vander Wall, 1990). For these experiments, the term **cache strategy** will be used to refer to the proportion of scatter and larder hoarding, which can be reflected in the single measure "cache density" (seeds per cache including scatter and larder hoards).

Larder hoarding is more common in mammals that scatter hoard. It is used whenever food stores can be defended against pilferage since scatter hoarding requires a specialized memory, and a large time and energy investment. Merriam's are smaller than many of the sympatric competitors (listed above) and cannot defend larder hoards (Randall, 1994). Merriam's may even reserve the peak foraging time to colder periods (an exception to the rule of decreasing activity with decreasing temperature) to avoid encounters with the larger sympatric Chisel-toothed kangaroo rat (Dipodomys microps) (Kenagy, 1973).

Efficient scatter hoarding requires that individuals remember where they cached food, so that retrieval minimizes energy use and exposure to predation. Merriam's kangaroo rats have specialized memory systems (Jacobs, 1992; Barkley & Jacobs, 1998). The hippocampus, the brain structure required for retrieving scatter hoards (Sherry & Vaccarino, 1989), is larger in Merriam's, and their performance on spatial memory tasks in the laboratory is better than Dipodomys species that do not scatter hoard (Jacobs & Spencer, 1994; Barkley & Jacobs, in preparation).

Despite the Merriam's kangaroo rat specialization for scatter hoarding, there are inter- and intra-individual differences in the proportions of scatter hoarding and larder hoarding (Jenkins & Breck, 1998). Merriam's kangaroo rats shift from a predominately scatter hoarding to a predominately larder hoarding strategy if their scatter-hoarded food is stolen by a familiar competitor (Preston, 1998; Preston & Jacobs, in press) or if the level of available food is very low (Preston & Jacobs, unpublished observation). In field

experiments, the distance from the food resource to the home burrow also changes cache strategy. When given a provision of food, animals larder hoard in the home burrow if food is found close to the burrow. As the distance between their burrow and the provisioned food increases, subjects scatter hoard immediately or larder hoard temporarily in a nearby burrow, scattering the food once the provision is depleted (Daly et al., 1992b). Thus, although Merriam's normally scatter hoard, thereby avoiding catastrophic cache loss from the burrow, they do larder hoard when scatter hoarding is disadvantageous.

#### **I.4. The hypothesis**

Because cache loss, predation and food deprivation are all likely to increase stress, stress could directly determine whether an animal will scatter hoard or larder hoard. Stress is defined here as an intervening variable that is induced by external environmental conditions that are unfavorable or challenging to the organism and causes historically adaptive changes in the organism's physiology and behavior. Stress is often operationally measured with levels of circulating glucocorticoids since these hormones are released in response to typical stressors like hunger, danger, and unpredictability. According to the model, in the natural habitat of Merriam's kangaroo rats, stable individual differences in stress predicts inter-individual differences in cache strategy while temporary increases in stress (from predation, pilferage, and hunger) would predict intra-individual changes in

cache strategy. The following paragraphs summarize the hypothesized relationship between stress and cache strategy.

When competition is low and/or food is abundant (low stress), animals do not hoard food at all, avoiding the predation risk and metabolic demands of cache/recovery trips, and avoiding the cognitive demands of remembering the location of caches. This condition is unlikely to ever occur in the wild since hoarding species evolved in environments with unpredictable food supplies and competition from heterospecific and conspecific individuals. In the laboratory, there may be cases where animals do not hoard food since it is superabundant and since animal care staff and experimenters regularly disturb caches.

When there is a risk of pilferage and the food supply is not abundant (intermediate stress), animals scatter hoard, creating a reliable food supply. This is the typical, modal case in the field for animals that evolved, developed, and live in an environment with an unpredictable food supply, especially for species and individuals that cannot defend a larder. Intermediate basal levels of glucocorticoids may be characteristic of small species like Merriam's kangaroo rats that are subordinate to many of the sympatric species. Because scatter hoarding is energetically and cognitively more demanding than not hoarding or than larder hoarding, this condition may be diminished in the laboratory. Scatter hoarding may require training in the laboratory to maintain the motivation of animals that do not have autonomous control over caches and cache recovery.

When scatter hoards are at great risk pilferage, when predation outside the burrow is unusually high, or food is very scarce (high stress), animals will remain in the burrow and protect a larder hoard. A high basal level of circulating glucocorticoids may be characteristic of larger, more dominant heteromyid species like D. microps or D. heermanni. Merriam's kangaroo rats that are subordinate to most sympatric species. This forced larder hoarding in Merriam's kangaroo rats may not occur often in the field (at least an extreme case), and it may be more likely to occur in laboratory conditions where larder hoards in the home cage are more reliable than scatter hoards.

*Overall, a positive relationship between cache density and stress is predicted with no cache density at low levels of stress, low cache density at an intermediate level of stress (scatter hoarding) and a high cache density when stress is high (larder hoarding) (Fig I.1.). However, the far left end of the curve may be less likely in field conditions than in laboratory conditions, and the far right end of the curve may be more likely in laboratory than field conditions. Of course, Merriam's kangaroo rats are characterized by their use of scatter hoarding because this strategy is more effective in the field where they must compete against larger, sympatric species. When larder hoarding is more effective than scatter hoarding (in the field or the laboratory), animals will rely more on larder hoarding. For species that are specialized to do either, the strategy that is more effective at the time will prevail. Given what is currently known about the neuromodulatory effects of arousal on many cognitive tasks (e.g. Yerkes & Dodson, 1908; Cahill & McGaugh, 1996; McEwen & Sapolsky, 1995), one would also expect that*

*the peak levels of scatter hoarding would correspond with the optimal level of stress for memory. It would be adaptive if cache strategy changed with memory capacity such that the more memory-demanding strategy was used when memory was facilitated and vice versa.*

This model was tested with a series of experiments. These experiments can be roughly divided into two major groups: experiments that manipulated stress using a social competition stressor, and experiments that used the metabolic stressor of food deprivation.

## **I.5. General methods**

### **I.5.1. Study animals**

Subjects for most experiments were trapped in January, 2000, in Palm Desert, California, USA and April, 2000, in Palm Springs, California, USA. All subjects were maintained at the University of California at Berkeley animal facility. Animals were housed individually in sand-filled (3 cm depth) plastic cages (48 cm x 27 cm x 20 cm high), each equipped with a home burrow (plastic ABS pipe approximately 17 cm long with a 6 cm diameter opening). Animals were fed 60 g of mixed seeds and two commercial rodent chow blocks once a week and one piece of Romaine lettuce three times a week as a water source. All of the subjects trapped in 2000 were maintained on 12:12 light:dark cycle, with lights off at 1600 hours. All subjects were run during their dark phase.

### **I.5.2. Apparatus**

There were two basic apparatus types: a large and a small hoarding arena. There was one large hoarding arena that could be used to test one subject at a time. This arena was used for the two experiments in Chapter II. This arena was composed of four white opaque acrylic walls (76 x 76 x 31 cm high) with a clear Plexiglas top that left space along one edge for air. There were two housing chambers (25 x 10 x 31 cm high) attached to diagonal corners of the arena (Fig. I.2.a); these were used to house the competitor animal (“competitor tunnel”). The arena floor was composed of four identical caching plates. Each plate consisted of a 26 x 26 cm styrene grid, composed of 1 x 1 x 1.24 cm deep

squares, yielding a total of 676 square cells in each plate and 2704 in the whole arena.

The bottom of each plate was covered in wire-mesh screen. The entire plate was covered with sand for a total depth of approximately 2 cm. With this design, caches could be located by lifting the plates and draining the sand entirely until all seeds were visible.

There were multiple small hoarding arenas that could be placed adjacent to each other to run and videotape many subjects simultaneously. These arenas were used for all other experiments. These arenas were white opaque plastic guinea pig cages, 51 cm x 41 cm x 20 cm high (Fig. I.2.b) covered either with a regular wire cage lid, or a Plexiglas top that left space along one edge for air. These arenas were also fitted with styrene grid floors composed of 1 x 1 x 1.24 cm deep squares. There was a single sheet of grid material 25 cm x 32 cm for a total of 800 boxes per arena. Wire mesh was affixed to the bottom of the styrene so that sand could be drained through the floor, leaving the caches intact for data recording.

### **I.5.3. Cache trials**

In all small arena experiments, each cache trial consisted of one overnight period that the subjects were left in the cache arenas. The trial started approximately when the dark cycle started (0400 h) and went until after the dark cycle ended the next morning (1000 h).

Subjects were placed into the arenas in their home burrows at one end of the arenas. At the opposite end of the arenas, subjects were given 100, shelled sunflower seeds (or the weight in grams of an average batch of 100 seeds: 6.5g) in a plastic dish. Next to this dish each subject was also given one-half piece of Romaine lettuce as a water source.

The large arena was used for two experiments. In these experiments subjects were also given 100, shelled sunflower seeds, but they were only left in the arena for as long as it took them to cache (usually 1 h). Because of the short duration of the cache trial, lettuce was not available. The exact duration of each cache trial is described in detail for these two experiments in Chapter II.

#### **I.5.4. Cache data collection**

After each cache episode, the exact location of each seed was recorded. The following measures were recorded: number of seeds found in the home burrow (larder hoarded), number and exact coordinate location (an area of 1 cm<sup>2</sup>) of seeds found in the arena but under the sand (scatter hoarded), number of seeds in each scatter hoard (cache size), number of seeds not found (eaten), number of seeds found in the arena, above the sand (uncached), and total number of seeds not eaten, i.e. larder hoarded, scatter hoarded, and uncached (recovered).

The number and coordinate location of grid boxes that were uncovered or dug out was also counted. An “uncovered” box was defined as a box on the grid where sand was removed such that the white top of the box was visible, but the box was completely full of sand. Cases where sand was also removed from the box were scored as “dug out”. Most cases of uncovering and some cases of digging out (especially large areas of adjacent boxes) seemed to occur when the animal moved sand from one part of the arena to another, perhaps to fill or surround the burrow. Other cases of digging out (especially individual boxes) were in locations where the animal retrieved a cache. The number of

boxes uncovered or dug out was analyzed for each experiment, but without discriminating the former type from the latter and without a specific hypothesis as to the outcome. A more detailed study can be done with the data in the future, analyzing the overlap of cache locations and subsequent dig locations.

For many multiple-day experiments, all seeds were returned to their pre-counting locations, sand was poured over the scatter caches to a total depth of 2cm, 1 cm above the grid. Uncovered or dug out boxes were not retained after this because sand was spread evenly over the surface. Even for experiments that did not last for multiple days, in most cases, seeds were returned to their original locations and animals were given at least one opportunity to return to the caches. In the first food deprivation experiment, animals were not allowed to retrieve their caches and an unusual proportion of seeds uncached developed across trials. Thus, in all subsequent experiments, subjects were given a chance to retrieve their caches to maintaining motivation for future caching.

#### **I.5.5. Video coding of behavior**

In some experiments, the behavioral data from each cache trials was videotaped and coded. The first five minutes of each trial were coded and analyzed according to a behavioral ethogram (Preston, 1999) using the Event3\_02 program by James Ha (University of Washington, USA). All measures were collected as intervals, total time in seconds, and percent time of the first five minutes. Space-use was divided into time spent in each side of the arena, time in the small tunnel, and at the food dish. Eating, chewing, sand bathing, rearing, and looking into the competitor tunnel (Chapter II experiments

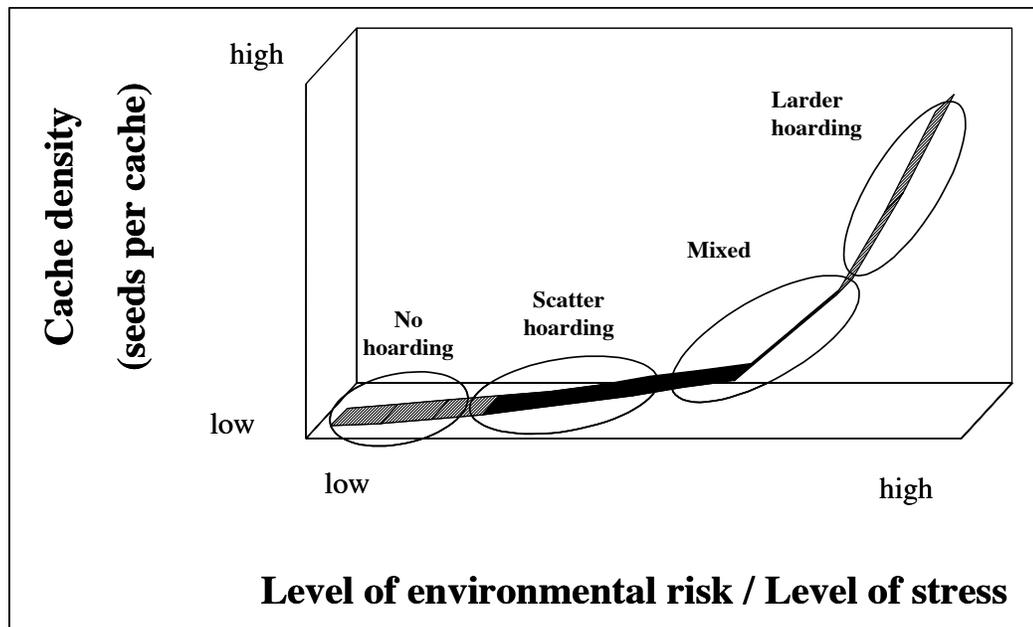
only) were recorded. Scratching, grooming, body shaking and tail biting were recorded and analyzed collectively as “displacement behaviors”.

#### **1.5.6. Food deprivation**

When food deprivation was required, subjects were temporarily removed from their home cages at the beginning of their dark phase, the sand was sifted to remove all food particles and replaced in the cage, all food in the home burrow was removed, and the subject was returned to the home cage. Each subject was given 1 g of oats and a 1-g piece of Romaine lettuce per day of food deprivation. Previous experience in the lab showed that animals do not reliably cache in the lab without food deprivation. Thus, in all experiments where the effect of the independent variable on scatter hoarding is in. Thus, for all experiments where the amount of food deprivation was not a variable (Chapters II and III), subjects were food-deprived for 24 h prior to the cache trial to induce hoarding. The remaining experiments were designed to systematically investigate the effects of food deprivation on hoarding and cache strategy.

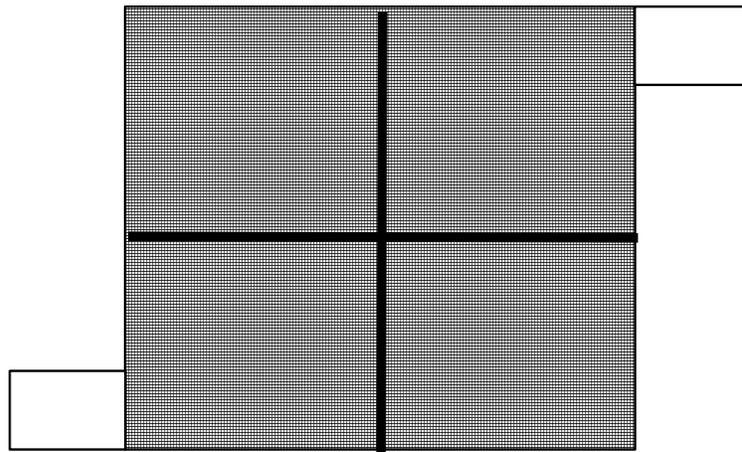
**Figure I.1**

Model of cache strategy where cache density (number of seeds per cache, including scatter and larder hoards) is predicted by the level of risk in the environment; risk in the environment also corresponds to the level of stress in the animal. Risk factors include pilferage, predation, and the scarcity of food. Hatched portions at the extremes of the function represent areas of the cache strategy curve that are more likely in laboratory conditions than in normal field conditions.



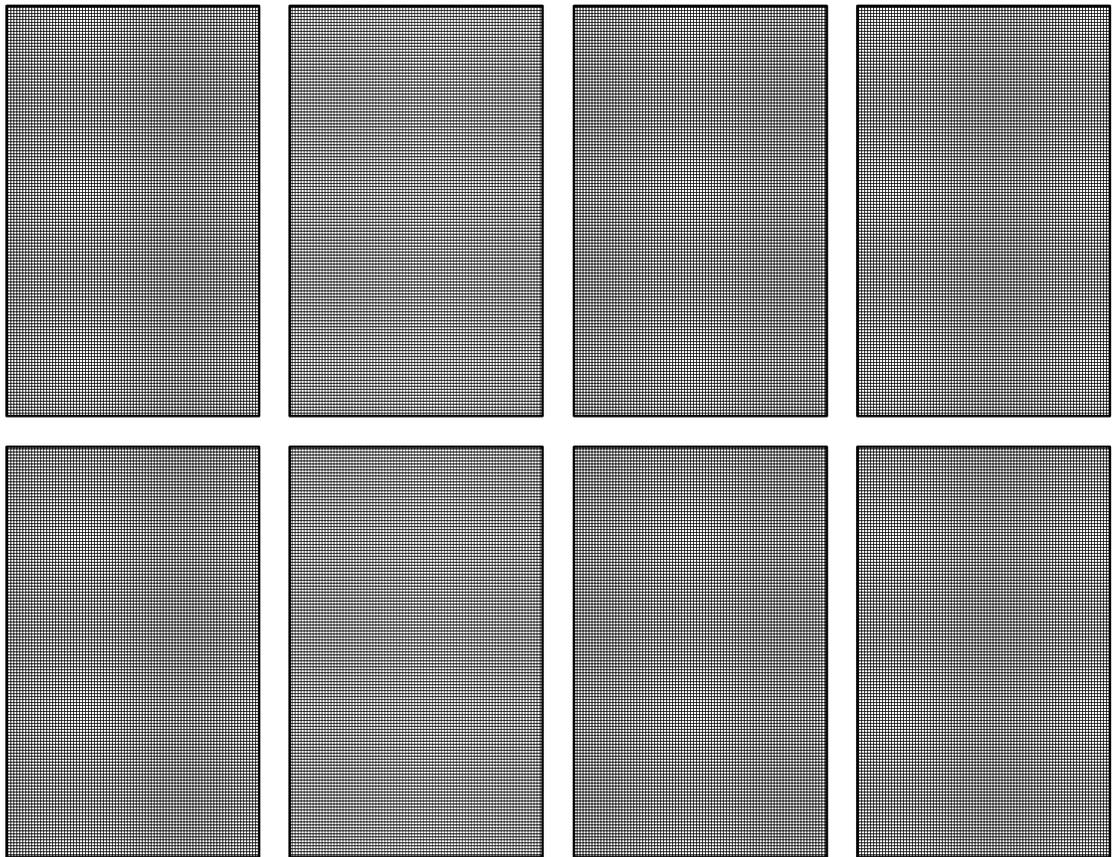
***FIGURE 1.2.a***

Schematic of the large arena (76 x 76 x 31 cm high). The inside cache area is composed of four square plates. There is a rectangular tunnel area attached on each side at a diagonal (“competitor tunnel”; 25 x 10 x 31 cm high). The exact number of cache boxes is not accurately rendered here. Arenas are not drawn to scale.



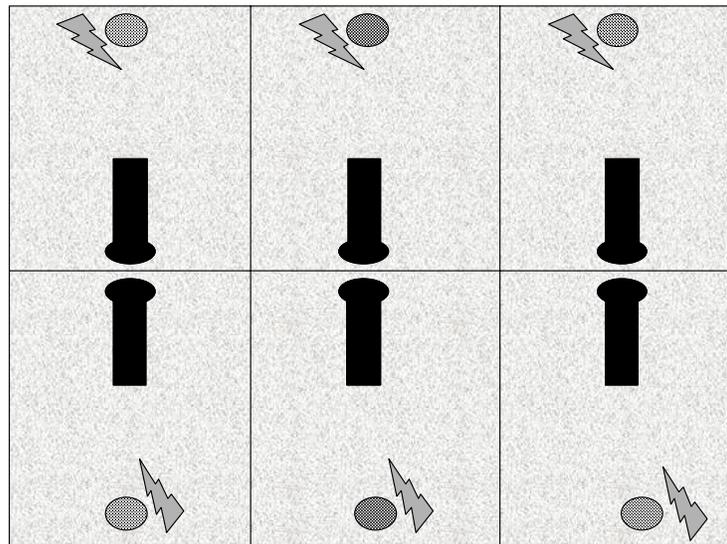
***FIGURE 1.2.b***

Schematic of eight small arenas (51 cm x 41 cm x 20 cm high). Cache areas are composed of a single plate, each contained within its own guinea pig cage. The exact number of cache boxes is not accurately rendered here. Arenas are not drawn to scale.



**FIGURE 1.3.**

Schematic of small arenas set up for a cache trial. Depicted are six arenas, placed adjacent to each other for simultaneous cache trials and videotaping. Each arena has a home burrow (black shapes) at one end that is facing a food dish (checkered ovals) and half-piece of lettuce (gray lightning shapes) at the other end. Sand covers the arena 1cm above the grid so that the grid is not visible on the surface. Arenas are not drawn to scale.



# Chapter II

## Experiments 1 and 2: The Effect of Heterospecific Presence and Pilferage on Cache Location Preference

### II.1. Introduction to social effects on caching

Social effects on foraging and caching have been examined in many food-storing bird species, but in few food-storing mammals. In rodents, laboratory rats cache less in group situations than when alone (Miller & Postman, 1946; Denenberg, 1952). Willow tits (Parus montanus) cache closer to the feeder when alone than in the presence of conspecifics, and dominant individuals cache closer to the food source and more quickly than subordinates (Lahti et al., 1998). Black-capped chickadees (Parus atricapillus) cache less when conspecifics are present than when they are alone (Stone & Baker, 1989). Coal tits (Parus ater) cache less when a neighbor is within 5 m (Brotons, 2000). Carolina chickadees (Poecile carolinensis) accumulate more mass over the day when in a flock compared to when alone (regardless of dominance; Pravosudov & Lucas, 2000). Overall, there is less caching by individuals when conspecifics are present.

This effect can also be seen at the moment of the cache decision. Many bird species abort caching in the presence of potential thieves (Kallander, 1978, James &

Verbeek, 1983, Burnell & Tomback, 1985) and grey squirrels (Sciurus carolinensis) engage in more false caching in the presence of an observer (Steele, in preparation). Bank voles (Clethrionomys glareolus) redistribute their caches into an unpreferred portion of the apparatus to avoid an introduced animal in the preferred portion, even though their nest is left in the preferred territory (Hansson, 1986).

These behaviors may be adaptive if pilferage is more likely when there was an observer of the cache episode. In a series of comparative experiments, Pinyon jays (Gymnorhinus cyanocephalus) ate more seeds before starting to cache in the presence of a conspecific observer and began caching sooner if paired with a more dominant individual. Observers remembered the area of the partner's cache. Group-living Mexican jay (Aphelocoma ultramarina) observers retrieved caches as well as the cacher for a two-day interval. These data suggest that observation facilitates pilferage. These jay species live in groups, and the effect need not generalize to solitary animals. Solitary Clark's nutcracker (Nucifraga columbiana) observers only benefit from observation for one day and are outperformed by the cachers. Thus, social living may increase information processing of observational information (Bednekoff & Balda, 1996). There is also anecdotal evidence of a cache observer immediately digging up the cache of a focal Merriam's kangaroo rat (Daly et al., 1992b), suggesting that being observed while caching is still a detrimental for solitary cachers.

Because Merriam's kangaroo rats live in overlapping territories, the risk of pilferage is likely to be a salient variable that increases with the density of competitors in

the area and decreases with the supply of food. Population density and food supply are also highly interrelated since the density of competitors is constrained at the upper limit only by the amount of food and access to available mates. A wide variety of rodent species pilfer the caches of Merriam's. In one experiment, all of the nocturnal rodent species on the study site were determined through fecal analysis to have pilfered the provisioned Merriam's (Daly et al., 1992b). Correlative field data and a systematic laboratory investigation of voles support the hypothesis that foraging increases with the density of conspecifics (Litvinov & Vasil'ev, 1973; Mappes, 1998, respectively).

Very little is known about how animals assess the risk of pilferage in their environment. In group-living animals, or when home ranges overlap, individuals should have accurate information on the density of competitors in the territory. Through caching and recovery, individuals have direct information regarding the rate of pilferage. Thus theoretically, animals could use indirect cues of competition such as number of sightings of competitors, scent cues, burrows etc., or they could use the direct cue of pilfered caches.

In my prior laboratory studies, cache strategy was not affected when two Merriam's kangaroo rats were placed in close proximity with each other (Preston & Jacobs, in press). In these experiments, animals went from caching alone, to caching with another conspecific on the opposite side of a wire-mesh barrier. Because there was a barrier separating the animals, and because the caching took place over a relatively small area (half of a small arena), this manipulation may not have mimicked field conditions of

increasing density closely enough to elicit a change. When subjects experienced pilferage from a proximate and familiar neighbor, cache strategy did change, but the change was only detectable on the first day after the pilferage (Preston & Jacobs, in press). These studies were not designed to be able to test for location-dependent changes in caching, since the arenas were uniform and pilferage was not restricted to an area. The following experiments were designed to see if area-restricted pilferage would cause individuals to more caches to an unpreferred area (as in Hansson, 1986, Stevens, 1984 and Hampton and Sherry, 1994). These experiments were also designed to further investigate the cues necessary for an animal to perceive the risk of pilferage.

## **II.2. Experiment 1 – Effects of heterospecific presence on cache place preference**

Experiment 1 determined whether area-restricted cache pilferage would affect cache location preference. A large single-subject arena was divided evenly into two completely different areas, to promote a preference for one half of the arena. One half was dimly lit and rich with landmarks, the other half was brightly lit and contained no landmarks. Because Merriam's kangaroo rats rely on landmarks for memory (Barkley & Jacobs, 1998) and rely on darkness for cover from predators (Daly et al., 1992a), it was predicted that animals would prefer to cache on the dark/rich side (hereafter referred to as the "rich side").

The Chisel-toothed kangaroo rat (Dipodomys microps) is a sympatric species that is dominant to and avoided by Merriam's in the field (Kenagy, 1973). This dominant species was introduced to the preferred side of the arena as a "competitor" (an animal that may compete with the subjects for resources) to try to change the subjects' preferred cache side. Behavior was also measured to separate and compare location side preference from cache side preference.

### **II.2.1. Study animals**

Subjects were wild-caught male Merriam's kangaroo rats run in the experiment in 1998. One was trapped near Palm Desert, CA in 1995, three were trapped near Reno, Nevada in 1996, and four were trapped in Palm Desert in 1997. The heterospecific competitors were Chisel-toothed kangaroo rats (Dipodomys microps) that were trapped in Reno, Nevada in October of 1996 and maintained in the same room with the same operating procedures as the Merriam's kangaroo rats. All of the subjects were housed since capture at San Francisco State University and/or the University of California, Berkeley. All subjects had experience caching in experimental arenas. None of the Merriam's kangaroo rats had prior experimental experience with a Chisel-toothed kangaroo rat, but some were wild-caught in the same territory and all were maintained in the same colony room as Great Basins, so they may have been familiar to each other (as individuals, or as a species). Animals were maintained on a reversed 12:12 light:dark cycle with lights on at 0900 hours. Subjects were food deprived for 12 h preceding the first day with approximately 1

g of oats and .3 g of lettuce (described in the general methods) to motivate the animals to cache.

### **II.2.2. Apparatus**

Tests were conducted in the large single-subject arena (described in general methods). For the entirety of this experiment, entrance to the two tunnels was blocked with wire mesh to prevent larder hoarding and to provide a housing area for the Chisel-toothed kangaroo rat competitor. The main arena was divided evenly by a black opaque barrier (61 cm x 28 cm x .32 cm wide) that extended three-fourths of the way across the arena so that animals could still move from one side of the arena to the other (Fig. II.1.). A plastic weigh boat was placed at the space between the two sides to dispense seeds. One side of the arena was richly decorated with a small bunch of artificial flowers, a rock, a small pine cone, a small opaque barrier and a small elbow of ABS tubing. This “rich” side of the arena also had a low level of illumination provided by a 25W red bulb attached to the table on the side of the arena that shone through the opaque plexiglass arena wall. The “bare” side of the arena did not contain any landmarks and was brightly lit with a 60W white light bulb attached to the table on the opposite side of the arena in a similar fashion. The side of the arena designated “rich” or “bare” was counterbalanced between subjects. To eliminate odor cues across trials, between each cache trial all sand was removed from the arena and sifted to remove non-sand particles, and all parts of the arena (including the barrier and the landmarks) were wiped down with alcohol and air dried.

### **II.2.3. Procedure**

Experiment 1 consisted of three trials, each separated by a 24 h break (Fig. II.1.). These were: premanipulation, manipulation and postmanipulation. During the one-day interval between experimental days, animals remained in home cages in the colony room on food deprivation.

For each trial, the subject was released in the arena, the side alternating across individuals and trials. One hundred, shelled sunflower seeds were available in the plastic dish. The first four subjects (two control, two manipulation) were given two hours to cache for each trial. Because virtually all caching was finished within one hour, the time allowed in the arena for the latter four subjects (two control, two manipulation) was reduced to one hour per condition.

After each trial, the location of all seeds was recorded (coordinate location of all scatter hoarded seeds, number of seeds left in the dish, and general location of all seeds left on the surface). All sand in the arena was sifted to remove particulate matter from the subject and the arena walls and landmarks were wiped down with alcohol to remove any scent cues from the subject and competitor. The number and location of boxes uncovered and dug up was also recorded. The side of cache preference was determined by the proportion of seeds placed on each side of the arena, with more than 50% of caches located on one side considered indicative of a preference. From video data (described in the General Methods), the side that the subject spent the majority of time (more than 50%) was considered the location side preference. These data were compared with the cache side preference data.

**Premanipulation** Each subject ( $N = 8$ ) was placed in the arena on a randomly chosen side and allowed to cache. After caching, he was removed and returned to the home cage with a new supply of oats and lettuce. The position of all seeds was recorded and all seeds were replaced for the next trial. The side of preference was determined by the proportion of seeds placed on each side of the arena, with more than 50% of caches located on one side considered indicative of a preference.

### **Manipulation**

*Manipulation subjects:* Caches were returned to their premanipulation locations. A Chisel-toothed kangaroo rat was placed in the competitor tunnel on the preferred side before the subject entered the arena. Each subject ( $N = 4$ ) was released into the arena on the opposite side as the previous trial and allowed to re-cache the all seeds from the premanipulation (cached and uncached). The trial lasted for thirty minutes, at which time the subject was removed and placed back into the home cage and given a new supply of oats and lettuce.

*Control subjects:* Each subject ( $N = 4$ ) was placed back into the arena for the second period, exactly as the manipulation subjects, but there were no competitor cues (presence of animal, visual and olfactory cues).

**Postmanipulation** This third trial (day 5 in the experiment) tested to see whether animals that switched “preferred” sides in the manipulation would retain their preference in the absence of the competitor. The procedure was identical to the premanipulation. Each subject ( $N = 8$ ) was given 100 new seeds, placed in the arena on the opposite side as the previous trial and given up to an hour to cache. There was no competitor for the manipulation subjects, and the cleaning of the arena removed all cues of the competitor’s prior presence.

#### **II.2.4. Analysis**

The alpha level was set at .05. All data were analyzed using repeated-measures ANOVAs with a three-level within-subjects factor (trial) and a between-subject factor (condition: control or manipulation). For some measures, side preference was quantified discretely (0 = bare, 1 = rich). Other cache measures were analyzed as a ratio of the variable on the preferred to the unpreferred side, usually based on the preference in Trial 1. Because this would often result in a 0 in the denominator, a 1 was added to both values (preferred and unpreferred) before taking the ratio (e.g. number of seeds on each side plus 1). These data are referred to in the results section as “ratio” data. There were only male subjects, so there was no analysis of gender.

#### **II.2.5. Results**

**Cache data** The majority of subjects preferred to cache on the rich side of the arena in the premanipulation (7/8 or 88%, trend for nonrandomness: Binomial test  $p = .07$ ). There was an increase in the use of and the preference for the bare side for caching across trials (Fig. II.2.). On average, subjects cached 14% (SD = 21) of their caches on the bare side in the premanipulation, 33% (SD = 33) in the manipulation, and 56% (SD = 48) in the postmanipulation ( $F(2,12) = 4.11, p = .04; r = -.449, p = .028$ ; Fig. II.3.); this increase in use of the bare side did not differ between control and manipulation subjects (interaction:  $F(2,12) = 1.26, n.s.$ ). There were no significant differences between control and manipulation subjects on the following measures: total number of caches, ratio of caches, number of seeds cached, or cache size (all measures:  $F(2,12) < 2.819, n.s.$ ).

**Behavioral data** Location side preference was strongly correlated with cache side preference ( $r = .519, p = .009$ ). Subjects generally started with a preference for the rich side in the premanipulation (7/8 or 88%; trend for nonrandomness: Binomial test  $p = .07$ ), and this preference shifted over time with up to 5/8 subjects preferring the bare side in the postmanipulation. As such, trial type was negatively correlated with side preference ( $r = -.433, p = .035$ ). There were some differences between the conditions; in general, manipulation subjects preferred the bare side more than the control subjects ( $F(1,6) = 6.00, p = .05$ ) and only manipulation subjects switched their location side preference when the Chisel-toothed kangaroo rat was introduced in the manipulation ( $t(6) = 3.00, p = .024$ ). But because of the low power and the fact that control subjects also

started to use the bare side in the postmanipulation, the interaction was not significant (trial-by-condition;  $F(2,24) = .176$ , n.s.).

Manipulation subjects spent more time than control subjects looking into the competitor's tunnel ( $F(2,12) = 5.80$ ,  $p = .05$ ), and the amount of time manipulation subjects spent looking into the competitor tunnel changed significantly more than control subjects from the premanipulation to the manipulation, measured as the absolute value of the manipulation minus the premanipulation (difference score =  $|(time2-time1)|$ );  $t(6) = -5.726$ ,  $p = .001$ ).

There were no significant differences in the percent time spent in the small tunnel, number of rears, time at the food dish, time chewing on non-edible items, or the number of displacement behaviors ( $F(2,12) < 1.01$ , n.s.).

## **II.2.6. Discussion**

There were no manipulation-dependent effects on cache place preference. The addition of the Chisel-toothed kangaroo rat to an adjacent compartment of the area did not deter the subjects' cache side preference. While the fact that there were only eight subjects may suggest that the experiment did not have enough statistical power to be significant, none of the cache measures even showed trends or indications that the manipulation subjects were deterred by the presence of the heterospecific. Therefore, I concluded that the manipulation was not sufficient to suggest a risk of pilferage to the subjects.

The manipulation subjects could see and smell the Chisel-toothed kangaroo rat, but they could not interact with the competitor. The behavioral data suggests that

manipulation subjects noticed the competitor was there, because these subjects looked into the tunnel more and changed their behavior towards the tunnel more than control subjects. In addition, the manipulation subjects seemed to avoid the competitor because they switched their side preference in the manipulation trial when the competitor was placed on their preferred cache side. The fact that the competitor was secured behind the wire mesh, may have rendered the threat innocuous. These results agree with previous experiments (Preston & Jacobs, in press) where actual pilferage associated with a competitor is required to elicit a change in caching. Experiment 2 (see below) addresses this issue by adding cache pilferage and strong scent cues of the competitor in the main compartment of the arena, on the preferred side.

During the postmanipulation, the caches from the manipulation were not replaced. This was done so that the final trial would mimic the first trial, rather than being an extension of the second. In this way, the experiment would determine if subjects would maintain or revert to their preference in an ostensibly new cache experience. These subjects were accustomed to having two-episode cache trials (cache and retrieve), thus, they may not have been disturbed by the fact that the manipulation caches were missing in the postmanipulation. However, the subjects may have perceived this change as a cache pilferage. From the premanipulation to the manipulation, most subjects increased the use of the unpreferred side in a graded fashion with an average of 20 percent change in cache preference and 23 percent in location preference. From the manipulation to the postmanipulation, three subjects stopped caching on the preferred side altogether, and

three decreased their new use of the unpreferred side. It is not likely that the reversal of strategy in the postmanipulation was due to the lack of the competitor because the changes in caching occurred for both manipulation subjects and control subjects. These facts suggest that from the premanipulation to the manipulation, subjects gradually started to use the other side (consistent with optimal cache placement theory of Tinbergen, 1967 and Clarkson et al., 1986). Then, the seed loss from the manipulation to the postmanipulation caused subjects to cease whichever cache strategy they had been using in the manipulation.

## **II.3. Experiment 2 – Effects of heterospecific competition on cache place preference**

Experiment 2 tested the effects of heterospecific pilferage on Merriam's kangaroo rat cache strategy and behavior. Specifically, this experiment tested to see if an individual would move caches to an unpreferred side of the arena if there were multiple cues of risk including: pilferage of caches from the preferred cache side, the presence of a heterospecific competitor in the tunnel adjacent to the preferred cache side, and scent cues of the competitor on the sand of the preferred cache side of the arena.

### **II.3.1. Study animals**

This experiment was run at two different times, with samples from two different populations. All subjects were wild-caught male Merriam's kangaroo rats. The first six subjects were from the same population as in Experiment 1 and this experiment was also run in the fall of 1998. The last four subjects were trapped in Palm Desert and Palm Springs, CA in 2000, and were run in the experiment in the spring of 2001. All were housed since capture at the University of California, Berkeley. All animals had experience caching in experimental arenas. The first six subjects were maintained on a reversed 12:12 light:dark cycle, with lights off at 0900 hours. The remaining four subjects were maintained on 12:12 light:dark cycle, with lights off at 1600 hours. All subjects were run during their dark phase.

### **II.3.2. Apparatus**

Tests were conducted in the large single-subject arena (described in general methods). For the entirety of this experiment, entrance to the two tunnels was blocked with wire mesh to prevent larder hoarding and to provide a housing area for the Chisel-toothed kangaroo rat competitor. The main arena was divided evenly by a black opaque barrier (61 cm x 28 cm x .32 cm wide) that extended three-fourths of the way across the arena so that animals could still move from one side of the arena to the other (Fig. II.3.). A plastic weigh boat was placed at the space between the two sides to dispense seeds. One side of the arena was richly decorated with a small bunch of artificial flowers, a rock, a small pine cone, a small opaque barrier and a small elbow of ABS tubing. This “rich” side of the arena also had a low level of illumination provided by a 25W red bulb attached to the table on the side of the arena that shone through the opaque plexiglass arena wall. The “bare” side of the arena did not contain any landmarks and was brightly lit with a 60W white light bulb attached to the table on the opposite side of the arena in a similar fashion. The side of the arena designated “rich” or “bare” was counterbalanced between subjects. To eliminate odor cues across trials, between each cache trial all sand was removed from the arena and sifted to remove non-sand particles, and all parts of the arena (including the barrier and the landmarks) were wiped down with alcohol and air dried.

### **II.3.2. Procedure**

The procedure for Experiment 2 was identical to Experiment 1, with the following exceptions (Fig. II.3.). For the manipulation subjects during the manipulation trial, not

only was there a competitor animal in the adjacent tunnel on the preferred side, but also, feces and soiled sand from the competitor were spread on the surface of the preferred side, and half of the caches were removed from the preferred side. If there was an odd number of caches, the larger number was removed, but a minimum of 30 seeds were left in the arena so that there would be enough seeds after the trial for statistical analysis. The removed seeds were placed with the competitor in the side tunnel where the subject could see but not access them.

The first six subjects were also in Experiment 1, and had a lot of experience caching in the lab; as a result, they often cached within 30 minutes of entering the arena. To minimize the time needed to run the each subject, each subject was checked in the premanipulation after 30 minutes. If there were no seeds in the plastic dish at this time, the subject was removed and given the same time for the remaining trials. If seeds remained in the dish at this time, the subject was given additional blocks of 30 minutes until he cached, up to 2 hours. After 2 hours he was removed and given another opportunity later in the day, or the subsequent day. In subsequent trials, all subjects were left in the arena for the time they required in the premanipulation.

#### **II.3.4. Analysis**

The alpha level was set at .05. All data were analyzed using repeated-measures ANOVAs with a three-level within-subjects factor (trial) and a between-subject factor (condition: control or manipulation). For some measures, side preference was quantified discretely (0 = bare, 1 = rich). Other cache measures were analyzed as a ratio of the variable on the

preferred to the unpreferred side, usually based on the preference in Trial 1. Because this would often result in a 0 in the denominator, a 1 was added to both values (preferred and unpreferred) before taking the ratio (e.g. number of seeds on each side plus 1). These data are referred to in the results section as “ratio” data. There were only male subjects, so there was no analysis of gender.

### **II.3.5. Results**

In the premanipulation, the vast majority of subjects preferred to cache on the dark, rich side of the area (5/7 control subjects, 7/7 manipulation subjects; nonrandom: Binomial test  $p = .01$ ); there were no differences between control and manipulation subjects in this preference ( $X^2(1) = 2.33$ , n.s.). One control subject only cached on the bare side, the other marginally cached more on the bare side, of all caches from the remaining 12 subjects, only one cache was placed on the bare side.

There were significant differences between control and manipulation subjects in the consistency of this preference. Control subjects but not manipulation subjects tended to only cache on one side for all three trials ( $X^2(1) = 4.67$ ,  $p = .03$ ; Fisher’s exact test (1-sided)  $p = .05$ ), conversely, only manipulation subjects increased their use of the unpreferred side from the premanipulation to the manipulation ( $X^2(1) = 7.14$ ,  $p < .01$ ; Fisher’s exact test (1-sided)  $p = .015$ ; Fig. II.4.). There was no difference between control and manipulation subjects in scatter hoard density or cache density across days (repeated measures ANOVA,  $F(2,24) < .492$ , n.s.). There was a decrease for all subjects in number of seeds cached and scatter hoard density in the manipulation ( $F(2,24) > 6.75$ ,  $p < .05$ ),

this was likely due to the design of the experiment, since subjects received new seeds on Trials the pre- and postmanipulation but not the manipulation.

Two of the seven manipulation subjects had seeds in their cheek pouches at the end of the manipulation trial. This is interesting from a strategy perspective since the subjects were not provided with a larger location and may have been attempting to avert further pilferage by caching in a more defensible location. There were also two control subjects that had seeds in their cheek pouches at the end of trials, but the onset of their use of cheek pouches did not correspond with the manipulation trial as it did for the manipulation subjects. It is also possible that the subjects were caching at the time of removal, but this does not seem likely since there do seem to be some individuals that are often found with seeds in their pouches, regardless of the circumstances. The power of this experiment was too small for these idiosyncrasies to be statistically significant ( $F(2,24) = .720$ , n.s.).

During the postmanipulation, when there were no cues of the heterospecific competitor, the majority of manipulation subjects maintained their new place preference while all of the control subjects maintained their original preference ( $\chi^2(1) = 5.6$   $p = .02$ ; Fisher's exact test (1-sided)  $p = .035$ ).

There was an unexplained difference between the early and later groups of subjects run in the experiment (1998 and 2001, respectively). Three of the first four manipulation subjects switched the preferred side of the arena entirely after experiencing the presence and pilferage of the competitor (the majority of seeds were cached on the

rich side before the manipulation, but the majority were cached on the bare side in the manipulation). In contrast, of the latter three subjects, only one put the majority of caches on the bare side, and even this subject did not put all of the caches on the bare side.

### **II.3.6. Discussion**

Whereas the single-variable manipulation of Experiment 1 had no perceptible effect on the subjects, there was a striking effect when multiple cues were used in combination. Most manipulation subjects changed their preferred cache side after experiencing cache loss paired with the presence of a dominant heterospecific competitor and the scent cues of the heterospecific in the arena. The manipulation subjects increased their use of the unpreferred side, while the control subjects continued to show a strong preference for the original side throughout.

The postmanipulation trial was intended to see if manipulation subjects would continue to show an altered preference once the heterospecific and the cues were gone. The results from this were heterogeneous, but the manipulation subjects significantly preferred to cache on a different side in the postmanipulation than they preferred in the premanipulation.

There are many possible reasons for the difference between the early and late subjects. The later subjects were from a different cohort, trapped later, in a different location, and with different lab experience. Some of the subjects in the early group were trapped at the same location as the Chisel-toothed kangaroo rats, thus, their prior field experience with the sympatric competitors may have increased their sensitivity. In the

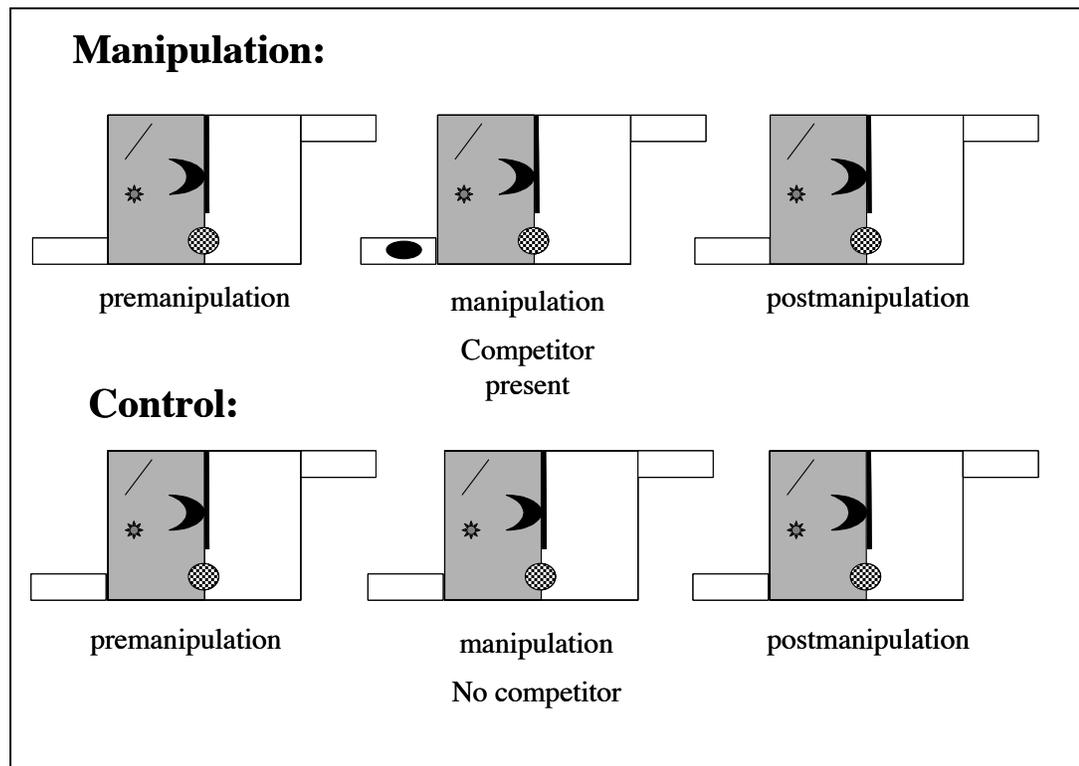
days that the later group was trapped in Palm Desert and Palm Springs, CA, no Chisel-toothed kangaroo rats were found in approximately 1000 trap settings. The lab experience of the two groups also differed. The early group participated in an extensive pilferage experiment that may have primed their perception of risk (Preston & Jacobs, in press). These subjects also participated in Experiment 1 where the competitor's presence did not affect caching, but the experience with the heterospecific competitor may have had a priming effect. The early group was also housed in the same colony room as the Chisel-toothed kangaroo rats and the communication of odors in the colony room may have had an effect. The role of prior experience with a competitor on the perception of risk should be investigated further.

#### **II.4. General discussion**

Both experiments attempted to alter cache place preference by introducing cues of pilfer risk. The results replicated those of Preston and Jacobs (in press) since subjects only moved caches after seeds were lost. An additional experiment with the presence and scent cues of the competitor should be done to confirm that the addition of the scent cues alone would not have been enough to effect a change.

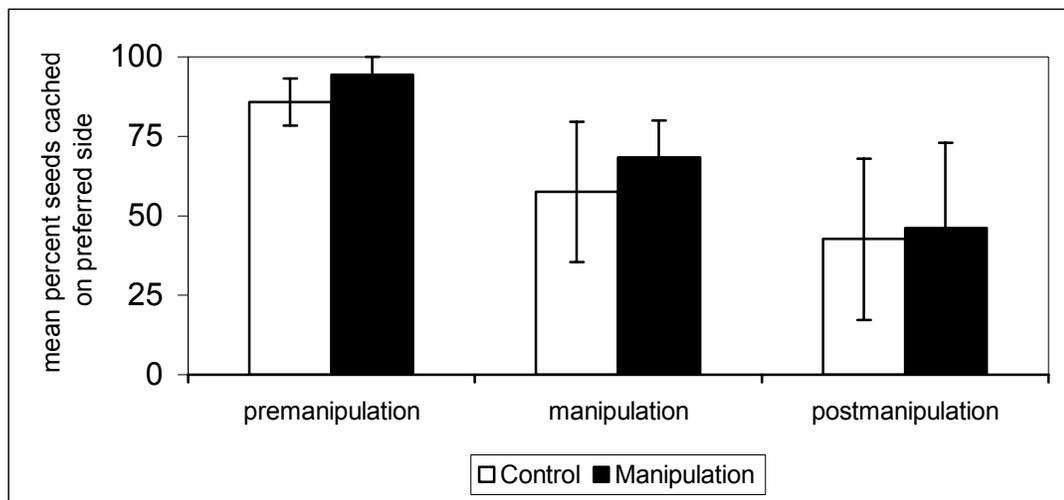
**Figure II.1.**

Schematic of Experiment 1. Subjects ran in three trials in the large arena, each separated by 36 h. The arena was divided into two equal portions, one dark with landmarks, one bright without landmarks. Checkered dish in bottom, center represents food dish. Competitor represented by black oval in the competitor tunnel of manipulation subjects only. Schematic is a sample where the manipulation subject preferred to cache on dark side, so competitor is placed in competitor tunnel adjacent to the dark side. Arenas are not drawn to scale.



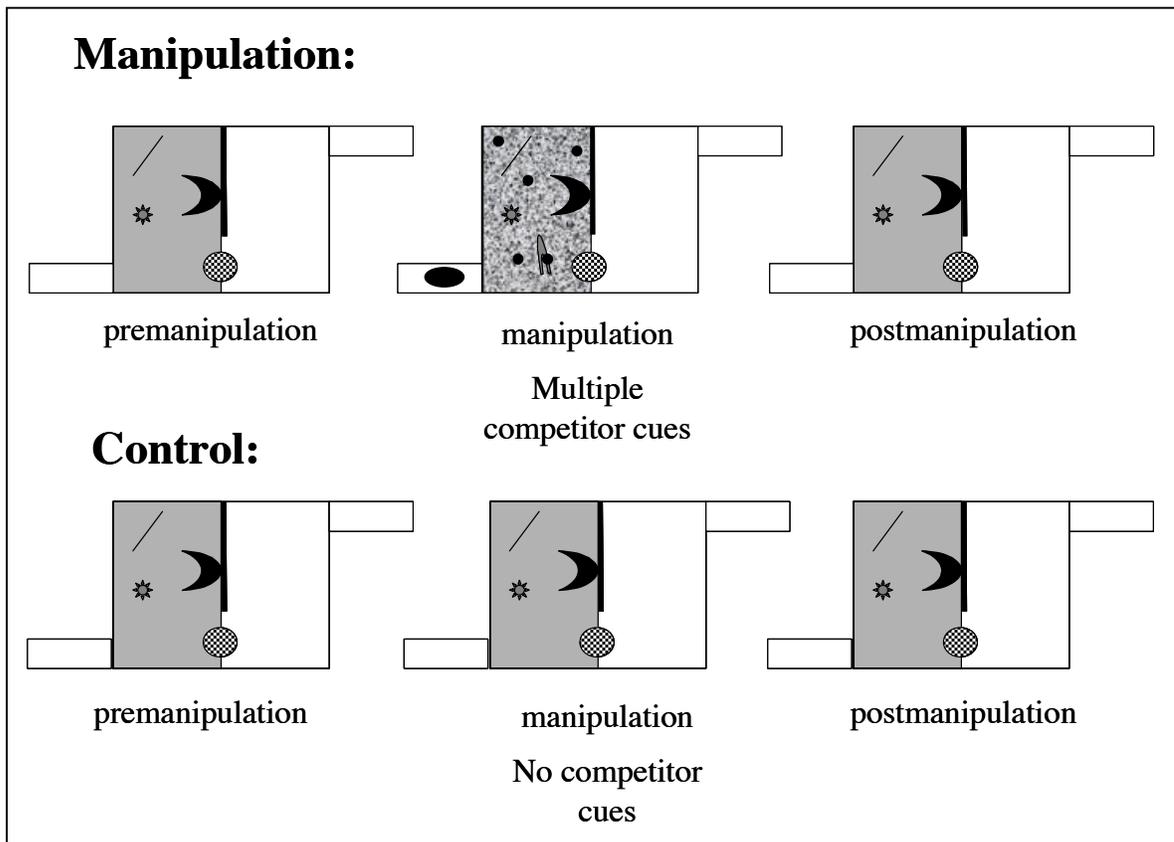
**Figure II.2.**

Experiment 1: Percentage of seeds placed on the preferred side over time (based on Trial 1 preferences, greater than 50% equals a preference).



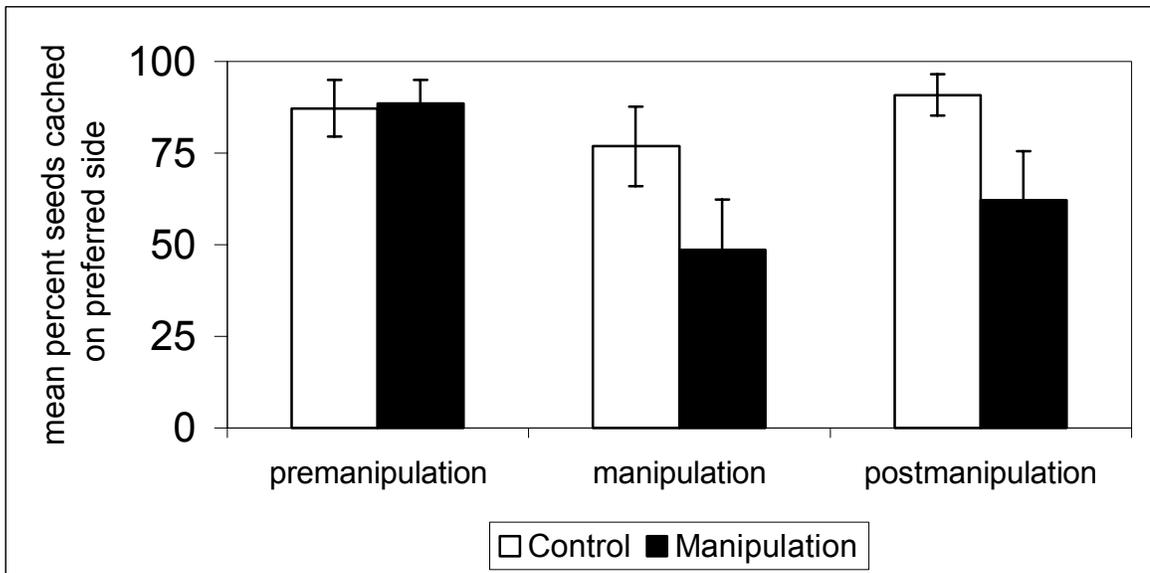
**Figure II.3.**

Schematic of Experiment 2. Subjects ran in three trials in the large arena, each separated by 36 h. Dark side of arena is represented in gray, shapes represent landmarks; bright side of the arena represented in white without landmarks. Checkered dish in bottom center represents food dish. Additional black circles on dark side represent scent cues of competitor spread on sand and pilfered caches. Competitor represented by black oval in the competitor tunnel of manipulation subjects only. Schematic is a sample where the manipulation subject preferred to cache on dark side, so competitor is placed in competitor tunnel adjacent to the dark side. Arenas are not drawn to scale.



**Figure II.4.**

Experiment 2: Percentage of seeds placed on the preferred side over time (based on Trial 1 preferences, greater than 50% equals a preference).



# Chapter III

## Experiments 2 and 3: The Effect of Being Pilfered and Pilfering

### Another on Cache Strategy

#### III.1. Introduction

**III.1.1. Effects of competitors on cache strategy** Optimal cache placement models have emphasized the need to space caches to avoid pilferage. The “spacing-out” hypothesis was first conceived and tested by Niko Tinbergen (1967), who theorized that prey species use camouflage in combination with high inter-individual spacing to avoid predation. Although the hypothesis was developed to deal with predator-prey interactions, it is relevant to this discussion for two reasons: 1) caches may be thought of as the prey of pilfering competitors and 2) the experimental test of his theory mimics cache pilferage conditions. He placed camouflaged eggs in patches of either high or low density and observed the subsequent amount of predation by wild carrion crows in each patch. More eggs from high-density patches were eaten by the crows, suggesting that crows use “area-restricted searching” to find the eggs. Furthermore, the crows remained in the high-density patches longer, seemingly because they had detected something about

the density of the patch and thus the probability of finding another egg. This provided evidence that spacing caches is an effective strategy to avoid pilferage.

The “spacing-out” theory has taken many shapes in the last thirty years as researchers have tried to model cache placement in food-storing animals while accounting for pilfer risk. At least three different but related cache-spacing models have been developed and tested since Tinbergen’s 1967 study. There is evidence for this strategy in at least three different food-storing species. Stapanian and Smith (1978) determined that the best strategy is to carry successive loads farther to decrease risk of pilferage while also minimizing the possibility of loss at the food source. This prediction was based on a previous model by the same authors (Stapanian & Smith, 1978), positing that the benefit of caching is equal to the ability to retrieve caches, both of which are proportional (not linearly) to the distance between caches. According to their model, as the distance between caches increases, the likelihood that a naïve competitor will give up an area-localized search before finding a second cache also increases (as in Tinbergen et al., 1967), thus discouraging multiple cache losses. Stapanian and Smith (1978) found evidence to support their theory in fox squirrels (*Sciurus niger*), where the percentage of artificial caches recovered decreased with the density at which they were hidden (the largest distance tested was 9.2 m between caches). Correspondingly, the grand mean cache distance (the mean of all of the individual squirrels’ mean distances between caches) from actual squirrel cache observations was 9.9 m.

Clarkson and colleagues made a similar prediction that the individual will try to cache near the source to decrease loss at the source, but in trying to decrease cache density will successively have to cache farther from the source (Clarkson et al., 1986). They tested their predictions with a field study of magpies (Pica pica). The results confirmed the major prediction because caches were placed at a lower density as the density-dependent loss of caches increased. These results further support the idea that cache spacing increases with the risk of pilferage.

In contrast to Stapanian and Smith (1978) and Clarkson et al. (1986), Sherry et al. (1982) predicted that an individual should carry early items farther and found support for their theory in marsh tits (Parus palustris).

In Merriam's kangaroo rats, artificial triplets of caches were placed in the field and tested for the rate of pilferage at different inter-cache distances (Daly et al., 1992b). Again supporting the "spacing-out" theory, the risk that all three caches in the triplet were pilfered decreased significantly at inter-cache distances greater than 2 m. In accordance, the average distance between caches observed in focal animals was between 2 and 3 meters.

These studies imply that a certain level of pilferage is going on continually and that the cache strategy of food-storing animals minimizes risk. Unfortunately, these hypotheses cannot account completely for the variance in cache strategy in Merriam's. For example, one expects that it would be more important to space out caches as the density of competitors increases (Jenkins & Peters, 1992), especially if the food supply is

widely spaced and an individual is unable to defend caches. If there are many competitors caching in overlapping areas, wide spacing causes the caches of different individuals to overlap, which may make it difficult for an individual to find its own caches, and re-introduces the risk of loss from an area-restricted search. Thus, the benefit of scatter hoarding may actually decrease as the density of competitors increases past a certain point (Jenkins, 1998).

**III.1.2. Questions that still need to be resolved** If individuals space caches optimally to avoid pilferage, how do they assess the environmental conditions and how do they determine the optimal distance? Is there an inductive process of adjusting distance according to the amount of pilferage on the last set of caches? Is there an innate algorithm that converts population density into an optimal nearest-neighbor distance? Do the animals detect the caches of other individuals in the ground and incorporate these into the decision (as would be posited by Jenkins inverted-U model for scatter hoarding)? These are important mechanistic questions, which need to be addressed along with the models.

There is evidence in food-storing birds that the direct experience of pilferage causes changes in cache strategy. Stevens (1984) found that marsh tits (Parus palustris) decrease caching in locations where they lost caches. The experiment did not determine, however, whether the birds became sensitized to the cache locations or to the distinctive substrate where caches were lost. Hampton and Sherry (1992) attempted to separate out these factors in black-capped chickadees (Parus atricapillus), and found that the birds learned to avoid the spatial location of caches where previous caches had been lost. They

also learned to reduce the amount of search time for caches in risky spatial locations. Although these animals are in some ways very different from Merriam's kangaroo rats, they are similar in two important respects: 1) Both the food-storing birds and mammals discussed here are specialized for storing food and they have common ultimate and proximate strategies (Sherry, 1985; Sherry et al., 1992). 2) Both of these animals are also susceptible to pilferage from many different species (Hampton & Sherry, 1992).

If pilferage is so rampant, why cache at all? Daly et al. (1992b) examined the benefits of caching with respect to the amount of pilferage and found that 5/9 of the animals that cached were pilfered, but none of the larder hoards were pilfered. It was the authors' contention that even in the cases where there was pilferage, the cache owner still benefited as much, if not more, from the caches. The owners retrieved at least one cache for every one that got pilfered and they were probably retrieving the caches for a greater time period than the pilferers (evidence of having eaten provisioned seeds was always seen in cache owners longer than in competitor animals). According to the authors, the strategy of caching was thus beneficial; scatter hoarding could be a "risk-sensitive" strategy to avoid the catastrophic loss that would result from pilferage of a larder hoard. Although none of the larders of these animals were pilfered during the observations, if they had been, the resulting loss would have been much more costly for the owner than for scatter hoards. It is important to note, however, that the individuals in that study were already caching at near-optimal nearest-neighbor distances. Thus, scatter hoarding may only be a successful strategy despite pilferage if optimal cache distances are utilized.

The following two experiments were conducted to find a simple multiple-subject experiment design that would mimic the effects from my Master's Thesis experiment, which showed a change in cache strategy after pilferage by a familiar conspecific (Preston, 1999; published as Preston & Jacobs, in press). The results from that experiment were novel and warranted further investigation, but the experimental design was very time and labor intensive, requiring daily seed checks and replacements for many months for a minimal number of subjects. Thus, it was desirable to find a simpler experimental design that could reproduce the effects on cache strategy. This would be especially beneficial for future work with on the proximate mechanism of caching, since many experiments could be done, relatively quickly to investigate the effect of hormone manipulations or to look for the endogenous release of hormones after a manipulation. The following two experiments looked for changes in cache strategy using the small arenas so that multiple subjects could be run at a time. They also used an experimental design that required less time in the arenas.

## **III.2. Experiment 3 – Effects of being pilfered on cache strategy**

### **III.2.1. Study animals**

The experiment was conducted at the University of California at Berkeley in April of 2001. Subjects were six wild-caught Merriam's kangaroo rats (2 females and 4 males) trapped in Palm Desert, CA, in January 2000, and Palm Springs, CA, in April 2000.

Conspecific competitors were drawn from a population of adults that were wild-caught as infants (born on the day of, or the day after trapping) in Palm Springs, CA, in April 2000 and raised in the laboratory. They were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

### **III.2.2. Apparatus**

Tests were conducted with six individuals at a time in the small multiple-subject arenas with wire lid tops. The room was lit with two 25 watt red bulbs. There were numerous extra-apparatus cues in visible in the room.

### **III.2.3. Procedure**

Two groups each consisting of three subjects ( $N=3$ ) ran in the experiment for four consecutive days. 24 h before the experiment started, all subjects were put on food deprivation (described in General Methods). On Day 1, each arena contained 6.5 g shelled sunflower seeds (weight of approximately 100 seeds) dyed with food coloring to facilitate visibility. Each day of the experiment, subjects were transferred in their home burrows from their home cages to the arena at approximately 4 pm and were allowed to cache overnight. At approximately 9 am the next day, subjects were removed from the arenas to their home cages while seeds and boxes were counted.

The experiment used a within-subjects design and controlled for the order effect by having the manipulation occur on a different day for the two groups of subjects (Fig. III.1.). For Group 1 animals, there was one premanipulation trial, followed by the manipulation trial, followed by two postmanipulation trials. For Group 2 animals, there

were two premanipulation trials, followed by a manipulation trial, followed by one postmanipulation trial. This way, if there were effects of the manipulation, the trial that the dependent variable changed would differ between Group 1 and Group 2 animals (see Fig. III.2.).

The competition manipulation was performed by a conspecific kangaroo rat (an adult individual caught in the field as an infant and raised in the lab). The competitor spent one night in the arena of the subject, ate and re-cached the subject's seeds, and slept in the subject's home jar. After the re-cached seeds were censused, they were returned to their new locations (where the competitor put them) and the feces and soiled sand of the competitor were spread on the surface of the sand to facilitate the perception of the manipulation. In two cases, the competitor did not disturb the caches of the subject, so the caches were moved by the experimenter to mimic the effects of other competitors.

#### **III.2.4. Data collection**

After each cache episode, the exact location of each seed was recorded. The following measures were recorded: number of seeds found in the home burrow (larder hoarded), number and exact coordinate location (an area of 1 cm<sup>2</sup>) of seeds found in the arena but under the sand (scatter hoarded), number of seeds in each scatter hoard (cache size), number of seeds not found (eaten), number of seeds found in the arena, above the sand (uncached) and total number of seeds not eaten, i.e. larder hoarded, scatter hoarded, and uncached (recovered). The number and coordinate location of grid boxes that were uncovered or dug out was also counted. An "uncovered" box was defined as a box on the

grid where sand was removed such that the white top of the box was visible, but the box was completely full of sand. Cases where sand was also removed from the box were scored as “dug out”. Video data were not coded.

### **III.2.5. Analysis**

All data were first analyzed with repeated-measures ANOVA with the four trials of the experiment as the within-subjects measure, and group as the between-subjects factor. Because of the small sample of each sex, the gender of the subject was not included as a between-subjects factor for this experiment. To increase power, variables that did not differ significantly across trials were analyzed with a three-level repeated measures ANOVA that combined the two postmanipulation trials of Group 1 and the two premanipulation trials of Group 2 into one measure, leaving all subjects with one premanipulation, manipulation, and postmanipulation value for analysis.

### **III.2.6. Results**

There was a main effect of day for the number of caches made ( $F(3,12) = 11.77$ ,  $p = .001$ ), with the number increasing linearly as the experiment progressed ( $F(1,4) = 19.91$ ,  $p = .01$ ). This effect is likely due to the increase in the total number of seeds available across days of the experiment as the number of caches is significantly correlated with the number of seeds recovered ( $r = .453$ ,  $p = .026$ ). There was also a main effect of day for the percent of seeds scatter hoarded ( $F(3,12) = 4.84$ ,  $p = .02$ ). This is due to a decrease in the percent scatter hoarded only on the last day of the experiment, compensated for by

non-significant increases in both larder hoarding and leaving seeds in the dish ( $F(3,12) < 2.273, p > .13$ ).

The only measures that showed the predicted directions of change were the boxes uncovered and dug out. There was a linear increase in the number of boxes uncovered across days ( $F(1,4) = 28.717, p = .006$ ) and a linear day-by-group interaction for the number of boxes dug up ( $F(1,4) = 9.03, p = .04$ ), with both measures peaking on the day that subjects experienced the manipulation, regardless of group. The number of boxes uncovered and dug out did not correlate with the number of caches or the percent of seeds cached ( $r < .26, p > .21$ ), but a finer-grained analysis of the exact locations of the boxes dug out may help determine the meaning of these behaviors.

The remaining cache measures were not statistically significant with this analysis ( $F(3,12) < 2.165, p > .15$ ). Strangely, the direction of the effects for many of these measures (density of scatter hoards, percent of seeds scatter or larder hoarded, ratio of scatter to larder seeds) were in the opposite direction from the prediction (see examples in Figs. III.3a and b). For each of these measures, the peak mean for Group 1 was on trial 3 while the peak mean for Group 2 was on trial 2; the pattern was supposed to be opposite to this with the mean peak for Group 1 on trial 2 and the mean peak for Group 2 on trial 3. The data were re-checked to confirm that the group numbers were accurately assigned. The higher power follow-up test that combined data into three levels (premanipulation, manipulation, postmanipulation) found this reversed pattern to be significant only for the density of scatter hoards ( $F(2,8) = 5.78, p = .03$ ).

### **III.2.7. Discussion**

As in previous experiments, the number of caches increased as the experiment progressed. However, there was a decrease in the percent scattered on the last day, and a concomitant increase in non-hoarding and larder hoarding; this may have been due to a superabundance of food. Subjects were given approximately 100 new seeds each day of the experiment. On average, subjects eat 20 seeds per day, thus, an excess of seeds accumulated over days of the experiment.

There were no manipulation-dependent effects on caching. The experiment did not have a large sample size, but even so, the graphical trends of most measures were not in the expected direction. Most were, in fact, in the opposite direction. The only logical explanation for this anomaly, besides random chance, is that the animals were most disturbed or affected when the manipulation was applied to the animal in the adjacent arena. For example, an increase in the activity level or vocalizations of neighbors who experienced pilferage and intrusion could have affected the cache behavior of neighboring individuals. Video analysis of the behavior, and replication with more power, may shed light on this paradox.

The main difference between this experiment and ones that did effect change in caching is the presence of the competitor. My Master's thesis and the second preference experiment in this dissertation achieved a pilferage-related change in caching only when the competitor was visible. In this experiment, a live conspecific performed the pilferage, there were scent cues of the competitor in the home jar of the subject and spread on the

sand of the arena, but the competitor was no longer present by the time the subject returned to find the pilfered seeds. Thus, kangaroo rats may require the presence of a competitor when caching before changing cache strategy.

These laboratory experiments may be inadequate for several reasons. Firstly, the size of the arena is small, so there is no cost for the subjects to search for the re-cached seeds of the competitor using olfaction or even digging up the whole arena. Secondly, most experiments are not run continually until the subjects have eaten all of the seeds that were originally cached, so they usually cannot return to caches that are made on the last day of an experiment. Thirdly, all caches made in the home cages in the colony room are lost bi-weekly when the cages are changed. Thus, cache loss overall in the lab is high, which may dilute the perception of pilferage as an experimental manipulation. Most importantly, in these pilferage experiments, the caches are dug up and replaced in new locations or eaten, but there are always enough seeds left to determine the new postmanipulation cache patterns. The very fact that the subjects have enough seeds to cache means that they have enough to eat, and thus, they may not be unduly disturbed by the manipulation. Overall, the relationship between cache effort and reward is not nearly as strict in the lab as in the field. An altered experimental design with reduced time or increased travel costs could address some of these issues.

### **III.3. Experiment 4 – Effects of pilfering another on cache strategy**

All previous experiments have examined how cache strategy changes after a subject's arena is intruded upon. This experiment was designed to see how a subject would react to intruding on the territory of another individual. The design also examined the response of animals when they returned to their original arena, which was intruded upon while they were in the arena of another. This provides some replication of the previous experiment as well as the new intrusion manipulation.

#### **III.3.1. Subjects**

The experiment was conducted at the University of California at Berkeley in April of 2001. I examined 16 wild-caught Merriam's kangaroo rats (8 females and 8 males), trapped in Palm Desert, CA, in January 2000, and Palm Springs, CA, in April 2000. Ten individuals were assigned to Group 1 (4 females and 6 males), six were assigned to group 2 (4 females and 2 males). All Group 1 individuals were run at the same time, and all Group 2 individuals were run at the same time. They were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

#### **III.3.2. Apparatus**

Tests were conducted in the small arenas with wire lid tops. The room was lit with two 25 watt red bulbs. There were numerous extra-apparatus cues in visible in the room.

#### **III.3.3 Procedure**

There were two groups run in this experiment. Group 1 (N = 10) ran in the full five-trial experiment while Group 2 ran in a shortened version of the experiment that only had two trials.

**Group 1** A total of ten animals (N = 10) ran in a small arena for a total of five trials (Fig III.4.). Half of were designated control (N = 5) and half were manipulation (N = 5).

For each trial, subjects were placed in a small arena in the afternoon (approximately 1600 h) with seeds, lettuce, and illumination from three 25-watt red bulbs. Three 100-watt bulbs were set on a timer to come on at 0400 h and off at 1600 h, retaining the light cycle of the colony room. Subjects cached overnight, and were removed from the arenas the next morning (approximately 1000 h) and placed in their home cages while seeds were censused. After censusing, all seeds were returned to their original locations, sand was poured on top of the grid to a total depth of 2 cm. Box locations that were uncovered or dug up on the previous trial were not preserved as sand was spread smoothly across the arena. For each trial, all subjects were given an additional half-piece of Romaine lettuce, but new seeds were only given for trials 1 and 4 (6.5 g, the average weight of 100 shelled seeds).

Trial 1 served as a baseline caching day. For trial 2, control subjects were placed in their original arenas from trial 1 to cache while manipulation subjects were switched such that each went into the arena of another manipulation subject. For trial 3, control subjects were returned to the arenas that they were in for both trial 1 and 2 while

manipulation subjects were returned to their original arenas from trial 1. Thus, control subjects continued to cache in the same arenas but manipulation subjects returned to their original arena, which had been intruded by a conspecific the prior trial. Trials 4 and 5 continued with all subjects in their original arenas to determine how long any cache effects would last. Because the majority of seeds were eaten by trial 4, all subjects were given additional seeds for this trial.

**Group 2** A total of 6 more animals (N=6) ran in the small arenas for a total of two trials the following week. Half were control subjects (N=3) and half were manipulation subjects (N=3). These two trials were exactly equivalent to trials 1 and 2 for Group 1. Group 2 was run to increase the power of the experiment by adding additional subjects, but on a shortened version of the experiment that only looked at the effect of intruding in the arena of another (trials 1 and 2). Thus, data could be combined across Groups 1 and 2 for the first two trials to see how intruding in the arena of a conspecific affected caching (effective N = 8 for each condition, control and manipulation).

#### **III.3.4. Data collection**

After each trial, the exact location of each seed was recorded. The following measures were recorded: number of seeds found in the home burrow (larder hoarded), number and exact coordinate location (an area of 1 cm<sup>2</sup>) of seeds found in the arena but under the sand (scatter hoarded), number of seeds in each scatter hoard (cache size), number of seeds not found (eaten), number of seeds found in the arena, above the sand (uncached),

and total number of seeds not eaten, i.e. larder hoarded, scatter hoarded, and uncached (recovered). The number and coordinate location of grid boxes that were uncovered or dug out was also counted. An “uncovered” box was defined as a box on the grid where sand was removed such that the white top of the box was visible, but the box was completely full of sand. Cases where sand was also removed from the box were scored as “dug out”. Video data were not coded.

### **III.3.5. Analysis**

All data were analyzed except where noted with repeated-measures ANOVA with trial as the within-subjects factor and condition, group, and sex as the between-subjects factors. For maximal power, one set of analyses were run on all subjects (Group 1 and Group 2; N=16) across trials 1 and 2 only to see if entering the arena of another individual had any effect on caching. Additional analyses were run on Group 1 only across all five trials of the experiment to see how caching changed from baseline, to intrusion, to being intruded, and back to baseline. There were no data on boxes uncovered or dug up from trial 1 of Group 1, therefore, these analyses are restricted to Group 2 subjects between trials 1 and 2, or to Group 2 subjects in trials 2 through 5.

### **III.3.6. Results**

**Groups 1 and 2; Effects of intrusion (day 1 to day 2)** Manipulation subjects only decreased their use of scatter hoarding when in the arena of another subject in trial 2, creating a trend for a trial-by-condition interaction for the ratio of scattered to larded seeds ( $F(1,9) = 4.22, p = .07$ ). Across both conditions, cache size decreased from trial 1

to trial 2 ( $F(1,9) = 5.67, p = .04$ ), but this effect did not persist when caches of only 1 seed were excluded ( $F(1,9) = 1.40, n.s.$ ). It is currently unknown whether boxes with a single seed occur as an intentional cache, as a seed left behind after retrieving the remainder of a cache, as an accidental placement of a seed that fell through the sand. Usually, data are analyzed with out without including these seeds as a cache, and the data are included here for both only because this is the first time that the data have yielded different results. Using either measure, there was a significant difference between subjects in Group 1 and Group 2, with subjects in Group 1 having smaller caches than Group 2 ( $F(1,9) > 7.61, p < .05$ ), and between females and males, with females having smaller caches than males ( $F(1,9) > 6.46, p < .05$ ).

The former effect is not caused by the latter since there are actually more females in Group 2. While most subjects in both experiments decreased cache size from trial 1 to trial 2 (14/16; Wilcoxon sign-rank test,  $Z = -2.59, p = .01$ ), the subjects in Group 1 had much larger decreases than those in Group 2 (average decrease Group 1 = 2.6, Group 2 = 0.49), though this difference was not significant ( $t(14) = 1.4, n.s.$ ). Mean cache size was larger in males than females for both trials of the experiment, however, in post-hoc tests, the difference between the males and females is due to the significantly larger cache size of males only in trial 1 and not in trial 2 ( $t(14) = -2.08, p < .05$ ;  $t(14) = -.73, n.s.$ , respectively). Including control and manipulation subjects, males decreased caching to the level of the females by trial 2; there was a trend for a sex-by-day interaction ( $F(1,14) = 3.05, p = .10$ ).

There was a significant decrease in the number of boxes uncovered from trial 1 to trial 2, and a significant trial-by-sex and trial-by-condition interaction for the number of boxes uncovered ( $F(1,3) > 284.41$ ,  $p < .001$ ). These effects should be regarded with caution since there are only 6 subjects in this experiment, only 2 of which are males. These effects are mostly due to the fact that one female on trial 1 uncovered an unusually high number of boxes (56 boxes,  $Z = 3.09$ ). By the coordinates of the boxes for this case, it is most likely that the sand was moved to cover the front of the home jar, which was adjacent to the area exposed; the change is not likely to be related to cache retrieval.

**Group 1 only; Full experiment effects (day 1 to day 5)** Cache density (a measure of cache strategy) changed significantly as a function of trial, trial-by-sex, trial-by-condition, and the three-way interaction of trial, sex and condition ( $F(4,24) > 7.41$ ,  $p < .001$ ). All three between-subject effects were also significant ( $F(1,6) > 83.38$ ,  $p < .001$ ). Females had higher cache densities than males (Female:  $N = 4$ ,  $M = 8.51$ ,  $SD = 6.66$ ; Male:  $N = 6$ ,  $M = 4.90$ ,  $SD = 1.98$ ) and manipulation subjects had lower cache densities than control subjects (manipulation:  $N = 6$ ,  $M = 5.54$ ,  $SD = 2.16$ ; control:  $N = 6$ ,  $M = 17.4$ ,  $SD = 7.89$ ).

These data were skewed by a single control female (DM404) that did not scatter hoard at all during the experiment; without this individual (acknowledging that eliminating a subject also reduces power), only the effect of trial was significant ( $F(4,24) = 3.79$ ,  $p = .017$ ). This particular female also contributed to a sex difference in the

percent of seeds larger hoarded (female greater than male:  $F(1,6) = 6.10, p = .048$ ) a sex difference in the percent scatter hoarded (female smaller than male:  $F(1,6) = 8.43, p = .027$ ) and a condition difference in the percent scatter hoarded (control smaller than manipulation:  $F(1,6) = 6.77, p = .041$ ).

The effect of trial for cache density is related to the effects of trial for number of caches ( $F(4,24) = 4.30, p = .009$ ) and percent of seeds left in the dish (uncached:  $F(4,24) = 4.70, p = .006$ ). Cache density and number of caches both had a statistically curvilinear relationship ( $F(1,6) = 26.03, p = .002$ ;  $F(1,6) = 9.10, p = .02$ , respectively), and all three measures had graphically curvilinear relationships with the lowest values by far in trial 3. There are two complimentary explanations for these effects of day, the changing number of seeds available across the experiment and the amount of time that had passed since the seeds were placed in the dish. A new allotment of seeds was given in trial 4 to replenish the dwindling supply from trial 1. The number of caches, but not the cache density or the percent uncached, significantly correlated with the total number of seeds recovered ( $r = .59, p < .001$ ;  $r < .15, n.s.$ , respectively) and all three tended to negatively correlate with the number of trials since the dish was filled (cache density:  $r = -.241, p = .09$ ; number of caches:  $r = -.22, p = .083$ ; percent uncached:  $r = -.22; p = .09$ ).

Cache size also changed significantly across days ( $F(4,24) = 4.80, p = .005$ ) due to a linear decrease across trials of the experiment ( $F(1,6) = 10.21, p = .019$ ). There were also between-subject effects for sex and condition ( $F(1,6) > 7.52, p < .05$ ). Females had smaller cache sizes than males (Female:  $N = 4, M = 3.42, SD = 2.25$ ; Male:  $N = 6, M =$

4.67, SD = 2.22) and manipulation subjects had larger cache sizes than control subjects (manipulation: N = 6, M = 4.67, SD = 1.66; control: N = 6, M = 3.68, SD = 2.73).

Cache size of control and manipulation subjects seemed to diverge on trial 3, when manipulation subjects returned to their original arenas, which had been disturbed by a conspecific. The main interaction effect of day and condition was not significant ( $F(4,24) = .245$ , n.s.), but there was a statistical trend for the two groups to differ on day 3 with manipulation subjects maintaining their day 2 cache sizes while control subjects continued to decrease ( $F(1,8) = 3.79$ ,  $p = .088$ ).

### **III.3.7. Discussion**

There were no striking effects of the manipulation on any measure of caching, suggesting that caching does not differ when an animal is in its own or in a competitor's arena. There were also no effects for Group 1 on trial 3 when they returned to their arena, which had been disturbed by a competitor. This suggests either that animals do not perceive pilfer risk when the competitor is not visible, or that they do not perceive pilfer risk when the seeds are simply re-cached and not removed, or that this particular method for producing pilfer risk in the laboratory is not effective. The manipulation may not have been salient to the individuals if food was too abundant, but this again invites the impossibility discussed in the previous experiment since enough seeds are needed for analysis, but few enough for the resource to be limited.

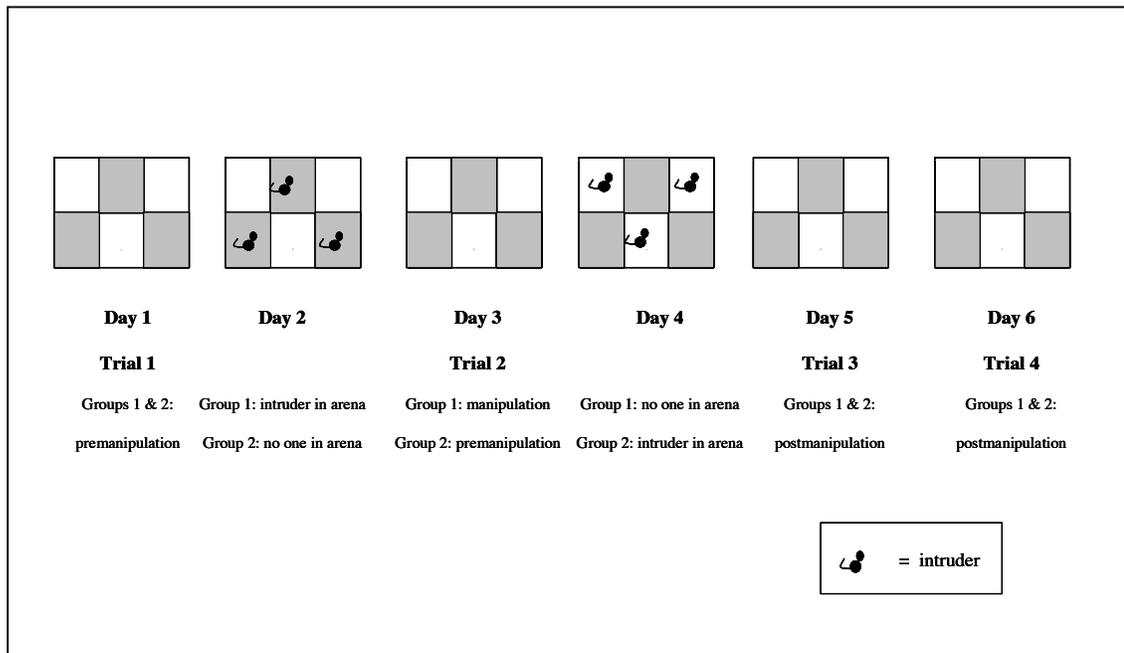
Perhaps this could be resolved with a design with two levels for each of two conditions: level of deprivation and opportunity to intrude. With such a 2 x 2 design,

subjects would be deprived or not deprived between caching and retrieval and then placed in the arena of another, or back into their original arena. Since food deprivation alone changes cache strategy (see next chapter), and since pilferage can cause a change in cache strategy, it would be valuable to combine the two variables into one experiment. This would also more realistically mimic natural conditions where pilferage is likely to correlate with a decrease in the availability of food.

There were many effects of trial that were related to both the total number of seeds that were available and to the number of trials since the dish was filled. Overtime, subjects increase caching, an effect that is replicated in almost every experiment to date. The increases in cache measures with the number of trials since the dish was filled support optimization models of scatter hoarding since the subjects gradually increase the distance over which they are caching and decrease the size of each cache.

**Figure III.1.**

Schematic of Experiment 3. Six subjects, three in each group, ran in an experiment with 4 cache trials, with two breaks. Arenas are placed adjacent to each other to run and be videotaped simultaneously. During the first break (Day 2), Group 1 arenas (gray boxes) were intruded by conspecifics, during the second break (Day 4), Group 1 arenas were not intruded. During the first break (Day 2), Group 2 arenas (white boxes) were not intruded, during the second break (Day 4), Group 2 animals were intruded by conspecifics. Intruders are represented by black figures in the arena. Arenas are not drawn to scale.



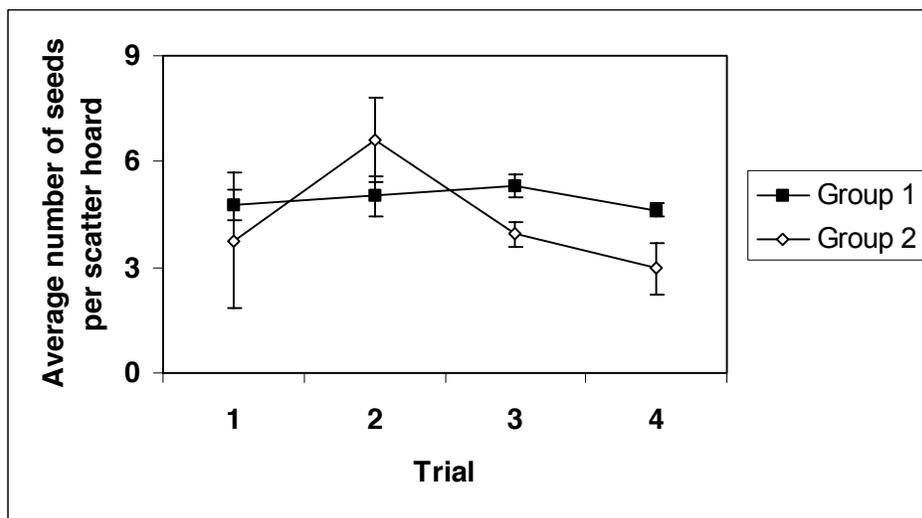
**Figure III.2.**

Experiment 3: Graphic illustration of a predicted experimental result for any given measure given the within-subjects design of the experiment (this graph depicting an increase in the measure).



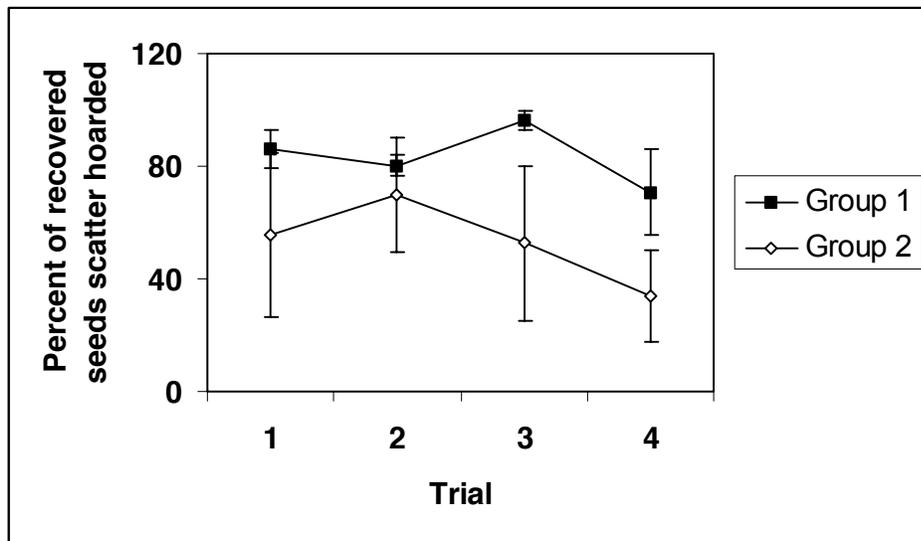
**Figure III.3.a.**

Actual data from Experiment 3 for the average cache size (average number of seeds per scatter hoard) across all four trials. Group 1 is represented by filled squares; Group 2 is represented by unfilled diamonds.



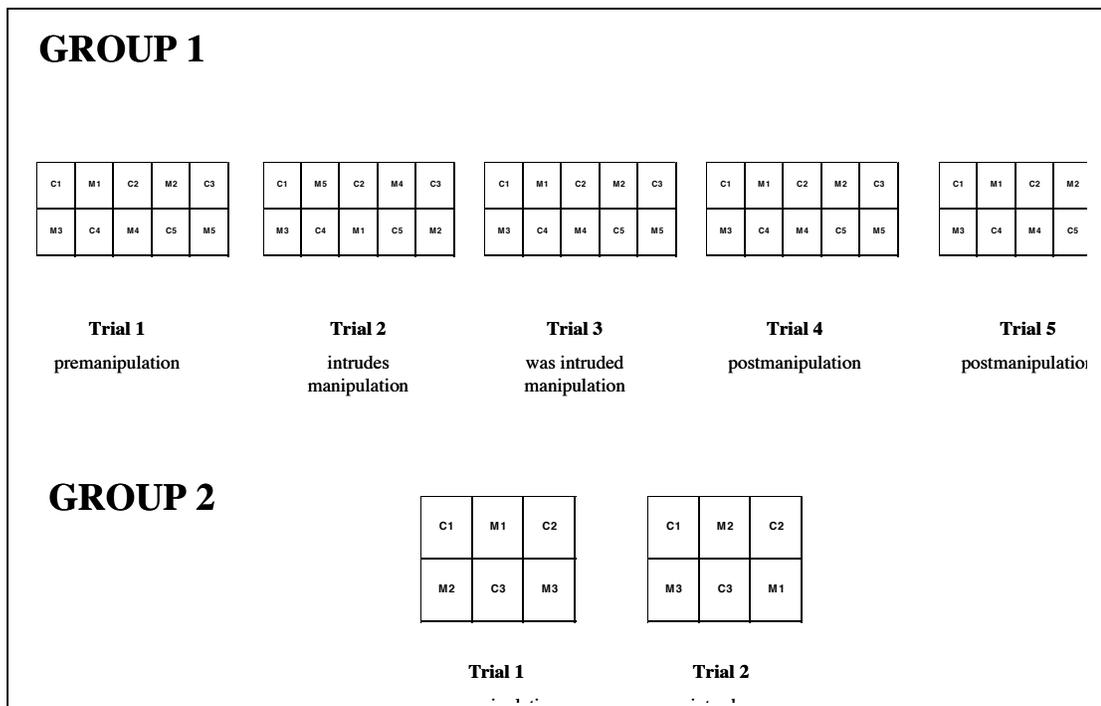
**Figure III.3.b.**

Actual data from Experiment 3 for the percent of recovered seeds scatter hoarded across all four trials. Group 1 is represented by filled squares; Group 2 is represented by unfilled diamonds.



**Figure III.4.**

Schematic of Experiment 4. Group 1 animals were in arenas for five consecutive 12 h cache trials. Group 2 animals ran in the experiment for two 12 h trials only. Each box represents a small arena, each group of boxes represents adjacent arenas where animals were run and videotaped simultaneously. Subjects are represented by their condition (C for control, M for manipulation) and their number. In trial 2 for both groups, the manipulation subjects switch arenas so that each is intruding in the arena of the another (intrudes manipulation). In trial 3 (Group 1 only), manipulation subjects return to their original arenas, which were intruded the previous day by another manipulation subject (was intruded manipulation). Control subjects are always in the same arena. Arenas are not drawn to scale.



**Table III.1.**

Experiment 4: Mean (SD) cache density for each sex and condition across all five days of the experiment for Group 1.

CONDITION	SEX (N)	1	2	3	4	5
Control	Male (4)	7.14 (2.24)	4.73 (1.71)	2.99 (1.21)	5.33 (1.69)	3.57 (1.02)
	Female (1)	22 (0.00)	22 (0.00)	7 (0.00)	11 (0.00)	25 (0.00)
Manipulation	Male (2)	7.22 (3.30)	4.19 (1.14)	4.38 (1.94)	5.83 (0.50)	4.29 (0.67)
	Female (3)	6.36 (0.68)	5.76 (3.82)	4.67 (1.91)	6.56 (2.68)	4.36 (1.03)

# Chapter IV

## **Experiments 5 and 6: Effects of Food Deprivation on Cache**

### **Strategy**

#### **IV.1. Introduction to the effects of food deprivation on hoarding across species**

Research on larder-hoarding species has found a relationship between the level of hoarding and the reproductive and metabolic state of the individual. Data across species are equivocal, with results that do not yet cohere, given the current models.

Hoarding in general appears to be an appetitive, rather than a consummatory behavior that takes future food needs into account (Urbano & Noble, 1981; McNamara & Whishaw, 1990; Whishaw & Kornelsen, 1993). It is not eliminated in rats even when the consummatory desire to eat is eliminated through illness or lipopolysaccharide treatment (Aubert et al., 1997). It is eliminated by neocortex lesions in the rat and the Syrian golden hamster, especially in the medial prefrontal area (Kolb & Whishaw, 1981; Kolb & Whishaw, 1983; Kolb & Whishaw, 1985; Whishaw & Oddie, 1989). This is thought to be

due to a decreased sensitivity to food cues, the level of risk in the environment, and previous experience (Whishaw & Oddie, 1989).

Siberian hamsters (Phodopus sungorus) increase their use of larder hoarding after a fast (Bartness, 1997; Bartness & Clein, 1994; Day et al., 1999; Wood & Bartness, 1996a; Wood & Bartness, 1996b). Syrian hamsters (Mesocricetus brandti) also increase hoarding after a fast (Wong & Jones, 1985), especially of high-fat foods (Lea & Tarpy, 1986); this increase occurs more in individuals with a high level of baseline hoarding, and the effect is attenuated by injections of leptin, a protein produced by fat that may be used to signal the body's level of fat to the hypothalamus (Schneider & Buckley, 2001). Montane voles (Microtus montanus) also increase hoarding of high-fat foods after a fast (Lanier et al., 1974). Laboratory rats increase hoarding with fasting, even though they do not generally store food when fed ad libitum (Fantino & Cabanac, 1980; Morgan et al., 1943; Cabanac & Swiergiel, 1989). One species of jird (Meriones unguiculatus) does not hoard in the laboratory when fed ad libitum, but does hoard after 1-2 weeks food restriction; this effect is greater in females than males and may mimic the seasonal changes in food supply and hoarding in the field (Nyby & Thiessen, 1980). On the contrary, another jird species (Meriones shawi) *eliminates* hoarding after 32 or 56h of food deprivation.

After a fast or food deprivation, many species increase eating to compensate for the lost body fat. This postfasting hyperphagia is exhibited by rats (Baker, 1955; Lawrence & Mason, 1955), house mice (Mus domesticus, Ross & Smith, 1983) deer

mice (Peromyscus maniculatus, Rowland et al., 1985) jirds (Meriones shawi, Demas & Bartness, 1999), and Mongolian gerbils (Meriones unguiculatus, Wong & Jones, 1985). However, many hamster species do not increase eating after a fast including Siberian hamsters (Bartness, 1997; Wood & Bartness, 1996a; Bartness et al., 1995; Day et al., 1999), Syrian hamsters (Rowland, 1982) and golden hamsters (Mesocricetus auratus, Rowland, 1983; Silverman & Zucker, 1976).

These data suggest a few alternative proximate mechanisms. Hoarding may be triggered by the loss of lipid stores in the body or a decrease in metabolic fuel utilization (Day et al., 1999). Supporting this theory, hoarding in female bank voles (Clethrionomys glareolus) is inversely correlated with weight (Mappes, 1998). Siberian hamsters preferentially store and eat high lipid content seeds (sunflower seeds), especially after repeated fasting (Day et al., 1999). Direct fat removal from Siberian hamsters through lipectomy causes an increase in hoarding until the remaining fat pads compensate for the loss. In rats, hoarding is so strictly correlated with weight loss below the set point that hoarding is used as an experimental indicator of an animal's set point (e.g. Michel & Cabanac, 1999).

In contrast to the body fat hypothesis, Demas and Bartness (1999) suggest a food availability hypothesis based on the hoarding of jirds. This is supported by the general finding that hoarding increases when foraging time is reduced (Bartness & Klein, 1994; Jenkins, 2000). In chickens, fasting-induced increases in corticosterone are eliminated when birds are simply allowed to view food without eating (Harvey et al., 1983).

However, the extinction of hoarding with deprivation in M. shawi suggests that the model does not apply across all species and that M. Shawi may have an alternative strategy with restricted food availability concentrating effort into the direct utilization and internal storage of energy, rather than into external storage (Demas & Bartness, 1999).

Given the complexity of results across species, it seems that species have hoarding and eating strategies that are specific to their metabolic systems (i.e. do they store fat well, do they efficiently break down stores into energy) that developed through evolution to be adaptive for their environmental conditions. For example, species that live in climates with non-productive winters and store fat well can hibernate or go into torpor to minimize energy demands and maximize the use of the fat stores. Other species use larders of food stored from spring through fall to survive during the winter.

Kangaroo rats live in areas where the productivity of the environment is less strictly seasonal than sporadic, and temperatures vary greatly within the day, but less so across days of the year than animals in more northern climates. These desert animals do not store fat, but rely on external stores of food. The type of storage (e.g. scatter or larder hoarding) depends on the net balance of the security of the cache type, predation risk, energy and time availability, and memory facility. Glucocorticoids play a special role in seasonal changes in fat metabolism (Lamberts et al, 1975) as well as in the facility of memory (de Quervain, 1998; Roozendaal et al., 1996), and thus may be involved in the proximate control of hoarding. Two food-deprivation experiments in this chapter tested the stress-based model of food storing in Merriam's kangaroo rats. Cache behavior and

circulating glucocorticoids were measured. All cache data will be described in this chapter while the glucocorticoids methods and data are presented in the next chapter.

## **IV.2. Experiment 5 – Effects of 0, 1, 2 days food deprivation on cache strategy**

### **IV.2.1. Study animals**

The experiment was conducted at the University of California at Berkeley in the fall of 2000. I examined 23 wild-caught Merriam's kangaroo rats (10 females and 13 males). Kangaroo rats were trapped in Palm Desert, CA in January of 2000, and Palm Springs CA, in April of 2000. They were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

### **IV.2.2. Apparatus**

Tests were conducted in the small arenas with wire lid tops. Six animals were run at a time. The room was lit with two 25 watt red bulbs. There were numerous extra-apparatus cues in visible in the room.

### **IV.2.3. Procedure**

This experiment used a within-subjects design with three levels of deprivation (0, 1, 2 days). Each condition was separated by a week and the order of the three conditions was counterbalanced across subjects. Each condition consisted of a 24 h cache trial in a small

arena with 100, shelled sunflower seeds. After the appropriate number of days of deprivation, subjects were taken from their home cages in their home burrows and placed into a small arena with a food dish and 2 g Romaine lettuce. The room was lit with three, 25 watt red bulbs during the dark hours. Three 100 watt white bulbs were connected to timers to come on at 0400 h and off at 1600 h, maintaining the light cycle in the colony room. Numerous extra-apparatus cues were available in the room. After a 24 h cache trial, subjects were removed from their arenas for blood collection and returned to the colony room. Caches from previous trials were not available in subsequent ones and animals were not given retrieval trials. Methods for blood collection are described in the next chapter.

#### **IV.2.4. Data collection**

After each cache episode, the exact location of each seed was recorded. The following measures were recorded: number of seeds found in the home burrow (larder hoarded), number and exact coordinate location (an area of 1 cm<sup>2</sup>) of seeds found in the arena but under the sand (scatter hoarded), number of seeds in each scatter hoard (cache size), number of seeds not found (eaten), number of seeds found in the arena, above the sand (uncached) and total number of seeds not eaten, i.e. larder hoarded, scatter hoarded, and uncached (recovered). The number and location of boxes uncovered or dug out was not recorded. Video data were not coded.

#### **IV.2.5. Analysis**

All data were analyzed with repeated-measures ANOVAs with the three deprivation conditions as the within-subjects measure and sex as a between-subjects factor. The alpha level was set at 0.05.

#### **IV.2.6. Results**

**Cache Results**        Animals increased the overall level of caching as deprivation increased (fewer uncached seeds  $F(2,42) = 16.196, p = .000$ ). Of all seeds given, the percent larder increased with deprivation ( $F(2,42) = 4.405, p = .018$ ). Of all seeds cached, males tended to use larder hoarding more than females, and tended to increase larder hoarding after less deprivation than females (interaction:  $F(2,10) = 3.23, p = .08$ ). Some males showed an inverted-U shaped change in cache density, with a low density at 0 and 2 days deprivation and a high density at 1 day of deprivation.

#### **IV.2.7. Discussion**

Cache strategy showed expected changes with food deprivation. There was more caching with more deprivation, a result that is consistently replicated in experiments with D. Merriami. There was also a change in cache strategy with an interesting sex difference where males increased larder hoarding earlier than females and some males showed an inverted-u shaped pattern with high density caching only for the middle, 1-day deprivation condition. This may be due to the general fact that male kangaroo rats tend to have higher levels of circulating glucocorticoids than females (see Chapter V). Thus, if both sexes have the same stress thresholds for cache strategy change, then males are closer to the point of change already, and need less stress to exhibit a change. This could

be tested with a longer period of food deprivation, which should cause a higher level of stress and a more curvilinear change in caching, especially in the females. Experiment 2 was designed to test this.

Alternatively, male individuals could be more sensitive to changes in the environment, yielding high basal levels of stress and a greater responsiveness to stress. This latter hypothesis could be tested with a stress-responsivity paradigm where animals are captured, handled, and retained for up to an hour, with repeated blood sampling (c.f. Wingfield, 1994).

### **IV.3. Experiment 6 – Effects of short- versus long-term deprivation on cache strategy**

This experiment was designed replicate the short-term deprivation results of the previous experiment, as well as to test the hypothesis that all subjects were not stressed enough by 2 days of deprivation in the Experiment 1 to show their peak levels of cache density. A long-term deprivation condition (> 1 week) was added to look for further increases in cache density.

#### **IV.3.1. Study animals**

The experiment was conducted at the University of California at Berkeley in April of 2001. I examined 12 wild-caught Merriam's kangaroo rats (6 females and 6 males). Kangaroo rats were trapped in Palm Desert, CA in January of 2000, and Palm Springs

CA, in April of 2000. They were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

### **IV.3.2. Apparatus**

Tests were conducted in the small arenas with wire lid tops. The room was lit with two 25 watt red bulbs. There were numerous extra-apparatus cues in visible in the room.

### **IV.3.3. Procedure**

This experiment had a mixed design (see Fig IV.8.). There was a between-subjects design for the short-term deprivation replication of the previous experiment (1 versus 2 days deprivation); Group 1 was deprived and given a cache trial one day later while Group 2 was deprived and given a cache trial two days later. There was a within-subjects design to compare short-term with long-term deprivation (<2 days or >7 days). Both groups were given a cache trial approximately 1 week after their short-term deprivation experiment. Therefore, each subject had two 24 h cache trials in the same small multiple-subject arena with 100, shelled sunflower seeds and 2 g Romaine lettuce each time. Blood collection procedures and data are discussed in Chapter V.

### **IV.2.4. Data collection**

After each trial, the exact location of each seed was recorded. The following measures were recorded: number of seeds found in the home burrow (larder hoarded), number and exact coordinate location (an area of 1 cm<sup>2</sup>) of seeds found in the arena but under the sand (scatter hoarded), number of seeds in each scatter hoard (cache size), number of

seeds not found (eaten), number of seeds found in the arena, above the sand (uncached), and total number of seeds not eaten, i.e. larder hoarded, scatter hoarded, and uncached (recovered). The number and coordinate location of grid boxes that were uncovered or dug out was not recorded in this experiment. Video data were not coded.

#### **IV.2.5. Analysis**

All data were analyzed except where noted with repeated-measures ANOVA with food deprivation condition as the within-subjects factor and sex as the between-subjects factor. The alpha level was set at 0.05.

#### **IV.3.6. Results**

For the between-subjects comparison of 1 to 2 days of deprivation, number of caches decreased from day 1 to day 2 ( $F(1,8) = 7.07, p = .029$ ); this response tended to be greater in females than in males (interaction:  $F(1,8) = 4.73, p = .06$ ). Larder hoarding of females only tended to decrease from 1 to 2 days deprivation (interaction:  $F(1,9) = 3.93, p = .079$ ).

For the within-subjects comparison of short- to long-term deprivation, subjects increased hoarding as deprivation increased (fewer uncached;  $F(1,10) = 5.82, p = .037$ ). Subjects tended to increase larder hoarding overall and to increase larder hoarding relative to scatter hoarding as deprivation increased from short to long term ( $F(1,10) > 3.54, p = .089$ ; nonparametric Wilcoxon  $Z = -1.86, p = .06$ ).

#### **IV.3.7. Discussion**

The longer-term deprivation (1 week) yielded very similar results to the short-term deprivation of the previous experiment (0-2 days). Subjects increased caching overall, and increased the use of larder hoarding over scatter hoarding. There was an unusual decrease in the number of caches from 1 to 2 days of deprivation, especially in females, which does not reflect an increase in the density of scatter hoards. Since this was analyzed as a between-subjects factor, a decrease may reflect the group effect caused by a few individuals. Females may have been more likely to decrease caching overall with 1 to 2 days deprivation even though they were more likely to larder hoard after 1 week of deprivation. The increase in larder hoarding in the long-term deprivation condition may reflect a loss of cache motivation since seeds were not returned after each cache episode and the long-term deprivation condition always came after the short-term deprivation (i.e. they were not counterbalanced). Subjects were given repeated trials (at least three) of cache and recovery as practice for the experiment so that they would be motivated to cache, but this may not have been enough. In addition, it would be unusual for the simple removal of caches by the experimenter to result in a change in caches strategy since prior experiments in this laboratory have not been able to elicit changes in strategy without a competitor present. A possible interaction between stress from food deprivation and the perception of pilferage could be investigated explicitly in an experiment like the one proposed in section III.3.7. Future research includes a third food deprivation experiment that will replicate this experiment but with the short- and long-term deprivation conditions counterbalanced; half of the subjects will experience short-term deprivation

before long-term deprivation and the remaining half will experience long-term deprivation before short-term deprivation.

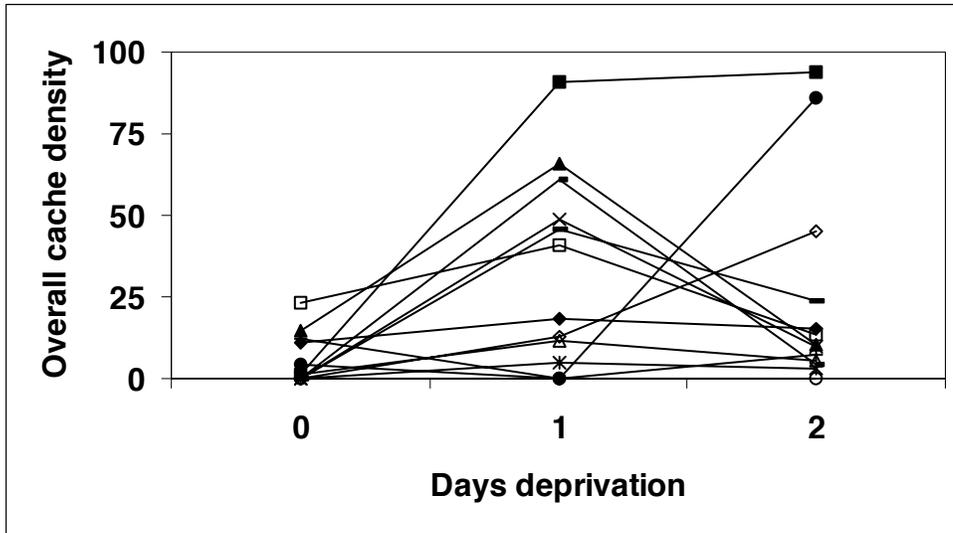
***Table IV.1.***

Experiment 6: Table showing three different orders subjects were run in to counterbalance the order effect of the three food deprivation conditions.

	<b>Condition 1</b>	<b>Condition 2</b>	<b>Condition 3</b>
<b>Order 1</b>	0 days deprivation	1 day deprivation	2 days deprivation
<b>Order 2</b>	1 day deprivation	2 days deprivation	0 days deprivation
<b>Order 3</b>	2 days deprivation	0 days deprivation	1 day deprivation

*Figure IV.1.a.*

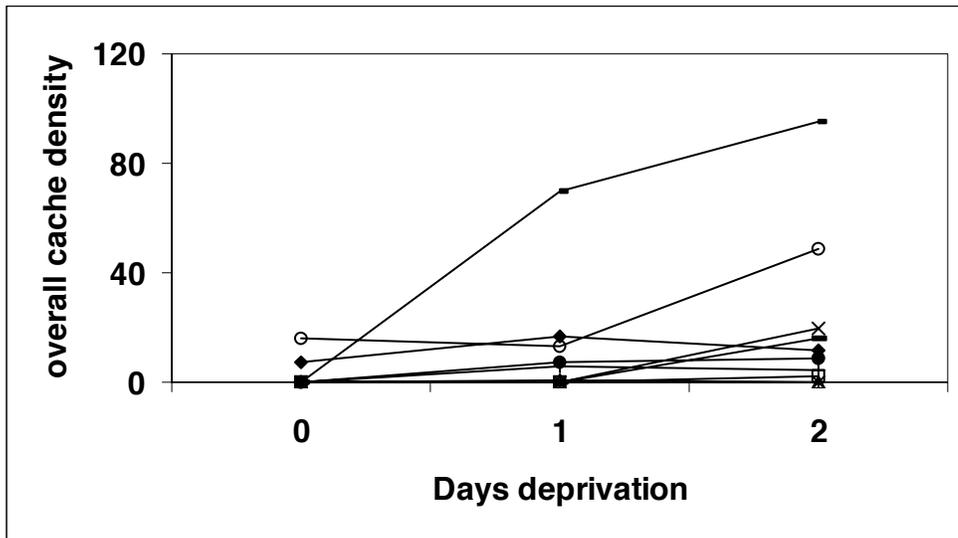
Experiment 5: Cache density for males across three conditions of food deprivation. Each line and accompanying symbol represents a different individual.



**Figure IV.1.b.**

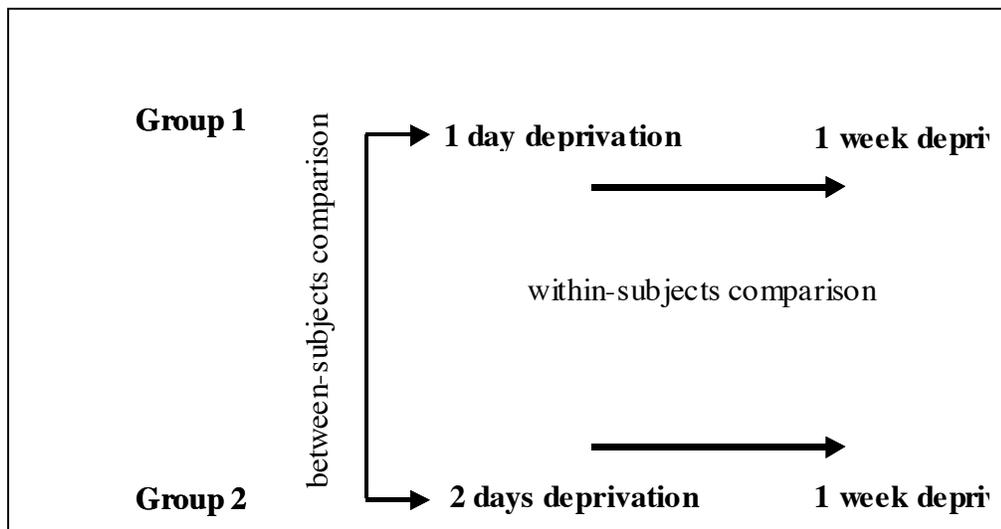
Experiment 5: Cache density for females across three conditions of food deprivation.

Each line and accompanying symbol represents a different individual.



**Figure IV.2.**

Schematic of Experiment 6. There was a between-subjects comparison between Group 1 animals that were food deprived for one day, and Group 2 animals that were food deprived for two days. There was a within-subjects comparison where each subject's short-term deprivation performance (1 or 2 days) was compared to its long-term deprivation performance (> 1 week).



# Chapter V

## **Experiments 7, 8, 9: Do Glucocorticoids Proximately Affect Cache Strategy?**

### **V.1. Introduction to the possible effect of hormone systems on caching**

Emotion physiologists have identified at least three separate but interacting subsystems for mediating the effects of arousal on cognition. There are distinct endogenous systems for responding to anticipatory stress (anxiety; benzodiazepine system, e.g. Medina et al., 1993), short-term stress (catecholamine system, e.g. Cahill & McGaugh, 1996), and long-term stress (glucocorticoid system, e.g. McEwen & Sapolsky, 1995). All three systems have been implicated in memory consolidation using primarily aversive learning tasks. Anxiety has also been implicated in decision-making in rodents with tasks measuring exploratory behavior and predator defense (e.g. Blanchard et al., 1993a; Griebel et al., 1996).

The benzodiazepine system (named for the class of pharmaceutical drugs that act at particular receptor sites) responds to the stress caused by the anticipation of danger, or anxiety. Benzodiazepine receptor agonists decrease anxiety while benzodiazepine

receptor antagonists increase anxiety. Benzodiazepine receptors are thought to interact with GABA-A receptors, increasing the post-synaptic potential of inhibitory synapses. In general, high levels of anxiety produce behaviors that are more risk-averse (e.g. Vasquez, 1994; Kotler, 1992; Ibrahim & Huntingford, 1989; Blanchard et al., 1993a; Blanchard et al., 1993b; Cao & Rodgers, 1997; Grewal et al., 1997; Griebel, et al., 1995; Daly et al., 1992a). Benzodiazepines are also known to affect memory in an inverted-U shaped fashion. Endogenous benzodiazepines released during training are thought to down-regulate (effectively decreasing) consolidation through GABA-A mechanisms in the basolateral amygdala, hippocampus and medial septum (Tomaz et al., 1993; Quirarte et al., 1997; Izquierdo et al., 1990). Benzodiazepines could thus be implicated in cache decision-making.

If environmental conditions are dangerous or anxiety-provoking, one would expect animals to avoid risk, and to rely on a cache strategy that is less memory dependent. For example, if there is increased predation or social conflict, it would be adaptive for animals to spend more time in the burrow and larder hoard rather than scatter hoard. Although larder hoarding is sometimes considered a risk prone strategy, in cases where leaving the burrow would endanger the survival of the animal, larder hoarding would be risk avoidant. However, if the anxiety comes from food competition and pilferage, then scatter hoarding may be the risk avoidant strategy, but this would not be adaptive if the memory of the animal is compromised by GABAergic inhibition. As evidence, in a study with laboratory rats, the injection of a benzodiazepine-receptor

agonist (diazepam) decreased hoarding in a dose-dependent fashion and impaired spatial navigation in a learning-set swimming pool task (McNamara & Whishaw, 1990).

The catecholamine system has also been researched extensively for its role in potentiating memory for emotionally arousing situations. In the fast-acting catecholamine system, epinephrine and norepinephrine are released by the adrenal cortex immediately in response to sympathetic arousal. These peripherally-released hormones activate the vagus nerve in the periphery, the efferents of which activates central  $\beta$ -noradrenergic receptors in the amygdala of the brain. This activation is thought to modulate the sensitivity of the amygdala to stimulation (Cahill & McGaugh, 1996).

Catecholamines may have profound influences on the consolidation of memory, but the contexts under which they are released are not likely to directly affect cache strategy. Caching is an anticipatory behavior that takes into account future needs (Urbano & Noble, 1981; McNamara & Whishaw, 1990; Whishaw & Kornelsen, 1993) and cache decisions must include information about the environmental conditions on a larger time scale than seconds to minutes; this information is unlikely to be reflected in a short-term hormone system. For example, if an animal sees a predator, a potential mate, or an abundant new food source, this may cause the release of catecholamines from the periphery and adaptively cause the animal to better remember where the predator, mate or food source was spotted, what it looked like and how to return to the site.

Catecholamines could, however, affect cache strategy indirectly. For example, seeing a competitor or discovering pilfered caches could arouse the animal, releasing

epinephrine and norepinephrine, which in turn in turn potentiate the animal's memory for the location or event. Thus, if pilferage is high in a certain area, then the animal may link the location to the pilferage and avoid caching there in the future. This could have been the mechanism for cache change in the place preference experiments in Chapter II. Similarly, if a majority of an animal's caches have been pilfered, there may be arousal that either potentiates memory and causes the animal to remember to use less scatter hoarding, or the arousal may be too high, reducing activity and impairing memory, which may have the same outcome of increasing larder hoarding. This latter case may also invoke the benzodiazepine and glucocorticoid systems and may have been the mechanism responsible for the results of my Master's experiment. The catecholamines system may directly affect cache strategy if the decision not to cache is taken as an example of strategy. Many species do not cache or abort caching in the presence of an observer (see section II.1.). In these cases, the presence of the observer may be a short-term stressor that arouses the animal too much to continue caching. Because this is the only likely direct effect of catecholamines on cache strategy, it would be difficult to study naturalistically in the lab. Animals are unlikely to cache during short-term stress, and it would be theoretically difficult to substantiate claim about a lack of caching. However, one do a cache observation test with half of the subjects receiving placebo and the other half receiving a  $\beta$ -blocking substance, such as propranolol. One would predict that caching would be suppressed only in the former condition. Thus, acting directly, catecholamines may terminate cache behavior, but they may indirectly affect cache

strategy by forming memories and associations with the outcomes of caching at different locations.

The slower-acting glucocorticoid (GC) system is also thought to be an endogenous modulator of memory (for reviews see Castellano et al., 1996; McEwen & Sapolsky, 1995). Stress increases activity of the hypothalamic-pituitary-adrenal (HPA) axis. In this system, the hypothalamus releases CRH, which stimulates the anterior pituitary to release ACTH, which in turn stimulates the secretion of glucocorticoids from the adrenal gland in the periphery (Axelrod & Reisine, 1984). The size of the glucocorticoid response depends on the duration and intensity of the stimulation from the hypothalamus and the pituitary, and on the effectiveness of the feedback mechanism whereby circulating glucocorticoids inhibit the activity of the HPA axis. Both catecholamines and glucocorticoids can increase memory performance from low to moderate doses, but impair memory with higher doses that mimic the levels released endogenously under stressful conditions (Cahill & McGaugh, 1998).

Circulating levels of glucocorticoids are likely to affect cache strategy. Conditions that would increase GCs include sustained high levels of predation, food shortages, food deprivation, and high social competition for mates or food. It would be adaptive in all of these conditions to change cache strategy. For example, abundant research shows that food deprivation increases both GC levels (see next section) and levels of hoarding, even in non-hoarding animals (see Chapter IV). Research in this dissertation shows that food deprivation increases hoarding per se, and also changes the proportion of scatter and

larger hoarding (see Chapter IV). Thus, there is circumstantial evidence that GCs control hoarding behavior. Moreover, theoretically, it would be adaptive for Merriam's kangaroo rats to scatter hoard when there is a moderate amount of stress from predation, food competition, and food deprivation (the usual case in the field, but not in the lab) because that is when their memory would be facilitated to remember the location of many, scattered caches. And when predation, food competition and food deprivation is very high, it is more costly for the animal to leave the burrow than to forage and search for caches, as such, larger hoarding would be a risk-averse strategy and would be adaptive since the memory of the animal would be impaired by the high levels of GCs .

Although all three hormone systems discussed above probably play a role in the complex interaction of emotion, decision making, and memory, this research focused on the role of glucocorticoids for multiple reasons. It is difficult to produce caching behavior with Merriam's kangaroo rats in the laboratory, and the release of either benzodiazepines or catecholamines was thought likely to inhibit cache behavior altogether. It is also difficult to find a naturalistic paradigm that could be used with Merriam's kangaroo rats that is known to release these substances endogenously, without a pharmacological injection. Catecholamines per se were thought to inhibit caching, and it is difficult to prove the existence of any mechanism that causes the lack of a behavior. Glucocorticoids were considered likely candidates for the direct influence on cache strategy, and they would be released in longer-term, natural stress conditions like food deprivation or competition, which are easy to replicate in the laboratory. Overall, due to

the time-course of their action, and their known role in food behavior, GCs were the most likely candidate for direct, proximate control of cache strategy.

### **V.1.1. Glucocorticoids and metabolism**

Glucocorticoids regulate energy allocation. They maintain homeostasis and respond to acute and chronic stress. When responding to stress, they emphasize the short-term needs of the individual by suppressing long-term anabolic processes such as reproduction, growth, and immunity, thereby increasing the catabolic breakdown and utilization of energy (Sapolsky, 1992; Siegel, 1980; Harvey et al., 1984). They alter osmoregulatory processes (May et al., 1990; Bentley, 1998) and act on the mammary tissue to prepare for and maintain lactation (Voogt et al., 1969). Glucocorticoids are lowest during mating and highest during lactation. They act directly in the hypothalamus to increase food intake and body mass. Conversely, high levels of fat result in a resistance to GC responsiveness to stress (see Kenagy et al., 1999).

In the bird literature, there have been extensive studies in the lab and the field of animal responses to common environmental challenges (e.g. low food availability from unusual storms, alpine winter cold, and desert summer heat). One of the most common stressors in the experimental literature is food deprivation. Restraint elicits a classical stress response in birds (e.g. Wingfield et al., 1982) and rodents (e.g. Conrad et al., 1996; Luine et al., 1994; Reburn & Wynne-Edwards, 2000). In caged birds, fasting is a potent stressor that elevates corticosterone to stress levels (Astheimer et al., 1992; Freeman et al., 1980). Food deprivation depletes fat reserves, which proportionally increases the

level of circulating corticosterone (Axelrod & Reisine, 1984; Cherel et al., 1987, Cherel et al., 1992; Wingfield, 1994) and these hormones can have metabolic as well as behavioral effects on the animals. Corticosterone increases may stimulate foraging, dispersal and the mobilization of energy to fuel these activities (Astheimer et al., 1992; Bray, 1993; Wingfield et al., 1997).

Corticosterone increases the metabolism of amino acids from muscle proteins (Veiga et al., 1978), which is beneficial for mobilizing energy to respond to short-term stressors, but, when stress is chronic, the elevation of corticosterone has deleterious effects including the suppression of memory, immune function, wasting of muscle tissue and cell death (especially in the hippocampus) (Sapolsky & McEwen, 1986; Sapolsky, 1992). In the short-term, these responses improve survival of the individual during food shortages (Astheimer et al., 1992), but they may cause an individual to terminate its breeding attempt or to abandon the nest, which reduces reproductive success (Silverin, 1986).

The net effect of these forces seems to depend upon the precise ecology and natural history of the species. In animals that are long-lived and are adapted to temperate breeding conditions, it would be adaptive to terminate a breeding attempt until the conditions are more favorable. In animals that are short-lived, have a high level of parental care, or live in extreme environments it would be more adaptive to persist in the breeding behavior despite poor conditions (Wingfield, 1994). The following experiments tested for a correlation between corticosterone and hoarding at the individual level by

assessing home cage caching behavior and tested for a group correlation between the level of food deprivation and corticosterone.

## **V.2. General method for hormone analysis**

All samples, except where noted, were taken between 1700 and 1900 h to avoid circadian variation and to replicate the levels of stress hormones in the field at the time of caching. Subjects were anesthetized with isoflurane in a gas chamber until they were no longer responsive to tail pinch. Blood was withdrawn from the tail vein with a 26-gauge needle (Henry Schein, Melville, NY) and drawn into a 70  $\mu$ l heparinized micropipette tube (Fisher, Santa Clara, CA). Once the tube was full, the needle was removed and the blood was placed into 1 ml Eppendorf tube (Fisher, Santa Clara, CA) and kept on ice. The subject was warmed manually and returned to the colony cage. After all samples were taken, the Eppendorf tubes were centrifuged at  $10,000 \times g$  in a 4° Celsius walk-in freezer. After 5 minutes of centrifugation, the supernatant plasma was drawn off, put in a new Eppendorf tube, and stored at -79° Celsius until assay.

Plasma corticosterone was determined using a radioimmunoassay (RIA) kit for corticosterone in rats and mice from ICN Biomedicals (Costa Mesa, CA). The intra- and interassay coefficients of variation are reported in each assay as an average percent overlapping in the standard samples provided in the kit.

## **V.3. Baseline sampling of corticosterone and a test of the assay for corticosterone**

In order to determine if the prepared blood collection and assay techniques were valid for use with kangaroo rats, a series of two baseline samples were taken from each adult in the colony, at two different times. From these baseline samples I could determine if the assay for rats and mice could detect corticosterone in the plasma of Merriam's kangaroo rats, what the average levels would be to later compare with stress-induced levels, what degree of individual variation there was in the colony to justify later studies of individual differences, and if the two samples taken approximately two weeks apart would produce similar values within an individual to determine the stability of the individual differences.

### **V.3.1. Study animals**

The experiment was conducted at the University of California at Berkeley in July of 2000. The first baseline samples were taken from each individual between July 10 and 14, 2000; there were 9 females and 10 males. The second baseline samples were taken from each individual between July 23 and 28, 2000; there were 9 females and 11 males. Samples were not collected either from females that were pregnant or nursing when trapped in April, or of their offspring that were born on the days surrounding trapping. Kangaroo rats were trapped in Palm Desert, CA in January of 2000, and Palm Springs CA, in April of 2000. They were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

### **V.3.2. Apparatus**

These were baseline samples, and as such, there was no testing apparatus. Subjects were removed directly from their home cages in the colony room for blood sampling. Animals

were housed in sand-filled (3 cm depth) plastic cages (48 cm x 27 cm x 20 cm high), each equipped with a home burrow (plastic ABS pipe approximately 17 cm long with a 6 cm diameter opening). Animals were fed 60 g of mixed seeds and two commercial rodent chow blocks once a week and one piece of Romaine lettuce three times a week as a water source.

### **V.3.3. Procedure**

The blood collection procedure took place in a testing room adjacent to the colony room. An induction chamber was filled with isoflurane, the timer was started, I entered the room and placed one animal from its home cage into the induction chamber to transport the animal to the procedure room. One in the procedure room, the induction chamber was connected through tubes to the isoflurane machine and the animal remained in the chamber until it appeared anesthetized. Then the animal was removed from the chamber, fitted with a nose cone to continue delivery of the isoflurane, and given a tail pinch to verify that the animal was anesthetized. The time that the animal was anesthetized (from the time I entered the colony room) was noted.

Blood was removed from the tail vein as described in the general methods above (section V.2.). After the full 70  $\mu$ l sample was collected, the time was noted, the blood was blown into an Eppendorf tube, labeled, and put on ice.

This procedure continued until all of the subjects for that day were sampled (no more than an hour after the collection of the first sample so that the blood would not be on ice too long before centrifugation). Approximately 6 to 10 animals were sampled each

time in this fashion. Since the subjects were sampled serially, there were multiple entrances into the colony room. The disturbance of the entrance was minimized by keeping the light level of the procedure room low, and keeping the noise of entering and leaving the room as low as possible. However, if the entrances into the room per se were disturbing for the animals, then these would not be perfect baseline samples. An order effect analysis could be done with the samples to determine if this was the case.

#### **V.3.4. Analysis**

Data were analyzed with ANOVA where gender and body weight were independent variables and the level of corticosterone was a dependent variable. This was done to see if there are any sex differences in the levels of corticosterone and if the level of corticosterone is related to the body weight of the animal.

To validate the blood collection procedure where animals were first anesthetized and then blood was withdrawn from the tail vein, an analysis was done to see if there was a positive correlation between the time it took to remove the sample and the level of corticosterone.

To validate the serial collection procedure where the colony room was disturbed multiple times (once for each subject collected that day), an analysis was done of the order effect. Each individual was assigned a number for the order that their sample was collected on a given day. These values were correlated with corticosterone values and an ANOVA was run with the order as the independent variable and the corticosterone value as the dependent variable.

### V.3.5. Results

The descriptive data indicate that there is individual variability in the samples. Across all 39 samples, the mean is 24.58  $\mu\text{g/ml}$  (SD = 9.8, SE = 1.57, range = 34.87). The data are slightly skewed (skewness = .15) with more values on the high end of the range than the low and platykurtotic (kurtosis = -1.03) without a wide, flat distribution rather than a bell-shape with a well-defined mean peak. However, there is not stability in these individual differences since the two baseline corticosterone samples did not correlate with each other ( $r = .29$ , n.s.).

In the first two baseline samples, the method and assay appeared validated, with values well within the expected range for an unstressed rodent (in ng/ml, Sample 1: mean (SD) = 28.47 (9.95); Sample 2: mean (SD) = 20.89 (8.37)). There was a sex difference in these values, with male values (Sample 1: mean (SD) = 31.32 (7.78); Sample 2: mean (SD) = M = 22.26 (9.34)) being higher than female values (Sample 1: mean (SD) = 25.31 (10.68); Sample 2: mean (SD) = 19.22 (7.18)). The direction of this sex difference was consistent across all assays but the power of any one was too small for significance. Although there is approximately one sample from each individual in each assay, a higher power test was run on the data combined across all data available (without meeting the independence requirement of ANOVA; there were approximately four samples from each individual); with this increased sample size, the sex difference was statistically significant ( $F(1,179) = 12.55$ ,  $p = .001$ ) with males having higher values ( $N = 96$ , mean (SD) = 30.20 (11.69)) than females ( $N = 85$ , M (SD) = 24.53 (9.57)). The body weight of males

is significantly higher than that of females (males:  $N = 55$ ,  $M (SD) = 42.08 (4.64)$ ; females:  $N = 46$ ,  $M (SD) = 37.90 (4.38)$ ;  $F (1,99) 21.43$ ,  $p < .001$ ). The correlation of weight with corticosterone, however, is not statistically significant ( $r = .157$ ,  $p = .09$ ).

There was not a significant correlation between the time required to withdraw the blood and the corticosterone values ( $r < -.234$ ,  $p > .05$ ), and the  $r$  value was unexpectedly negative. However, this measure does not include all of the time that the experimenter was in the room, only the time required to extract the blood from a single subject once anesthesia started. There also was not a relationship between the order that the animals were sampled and the level of corticosterone ( $r = .16$ , n.s.;  $F(8,30) = .297$ , n.s.); again the  $r$  value was negative. This latter measures the number of times that the colony room was disturbed before each individual's sample was taken, but it does not measure the amount of total disturbance time.

### **V.3.6. Discussion**

Based on the results of the baseline sample analysis it appears that the corticosterone assay for rats and mice will work sufficiently on Merriam's kangaroo rats. Based on the mean values for rats and mice, the values from the baseline samples would reflect a low baseline measure. Because these values also appear not to reflect stress levels, it is likely that the blood collection procedure was adequately rigorous to prevent handling stress from the procedure to affect the samples.

There was not a positive correlation between corticosterone and the time required to handle each animal before the sample was taken, or the number of entries into the

colony room that day. Indeed the direction of both Pearson correlation coefficients was negative. Thus, it seems that the blood collection procedure is adequate to prevent stress from the procedure from contaminating the baseline level of corticosterone in the blood (unless the first sample was also contaminated, setting a stress level as the minimum). The blood sampling was continued with the same protocol and behavioral paradigms were developed to test the stress-based mechanism for hoarding.

Males had higher weights and corticosterone levels, on average, than females. Applying these data to the model, one would expect that males and females would also have different cache strategies, the exact strategy difference depending on where on the stress curve each sex is at baseline and after a manipulation is applied. Since both corticosterone and home cage larder hoarding show individual variation, the relationship between stress and hoarding was first investigated with a simple test of individual variation in larder hoarding in the home cage.

This was followed by an experiment with a stress manipulation to try to change the position of each gender on the curve of stress and hoarding. Food deprivation was chosen as the manipulation because it has been proven to be a potent stressor for most species and one that does not require the sampling of blood during a stressful behavior (as is the case with a social competition manipulation).

#### **V.4. Experiment 7: Individual differences in cache strategy and corticosterone**

One way to study the relationship of stress hormones to caching is to examine individual differences in corticosterone under different conditions. In degus (Octodon degus), seasonal changes in cortisol are correlated positively with seasonal changes in body mass (during mating, both were lowest), but there is generally not a correlation at the individual level between weight and cortisol level (Kenagy et al. 1999). A correlation between body mass and glucocorticoid levels across seasons was found in yellow-bellied marmots (Marmota flaviventris, Armitage, 1991) and golden-mantled ground squirrels (Spermophilus saturatus, Boswell et al., 1994) and there was a correlation between individual weights and cortisol levels in lactating female meadow voles (Microtus pennsylvanicus, Boonstra & Boag, 1992).

One obviously varying individual behavior of our animals was the use of the burrow pipe to store food in the colony cages. In October and November of 2000, the amount of food larder hoarded in the home pipe was sampled eight times over three weeks (approximately every three days) to determine the variability of this behavior within and across individuals and genders. These data were also compared to the two baseline measures of corticosterone to determine if this measure of cache strategy could be predicted from the individuals' baseline level of circulating glucocorticoids. However, the larder data and the blood samples were not collected at the same time (they were approximately four months apart). A correlation is only expected if there was not great variability in stress between the time that the larder hoarding data and the two blood samples were collected.

#### **V.4.1. Study animals**

The experiment was conducted at the University of California at Berkeley from October 27 to November 16, 2000. I examined 28 wild-caught Merriam's kangaroo rats (15 females and 13 males). Kangaroo rats were trapped in Palm Desert, CA in January of 2000, and Palm Springs CA, in April of 2000. Two of the females and three of the males were individuals trapped as infants in Palm Springs site and raised in the laboratory. The remaining animals (13 females and 10 males) were trapped as adults. All animals were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

#### **V.4.2. Apparatus**

Tests were conducted in the animals' home cages in the colony room. Animals were housed individually in sand-filled (3 cm depth) plastic cages (48 cm x 27 cm x 20 cm high), each equipped with a home burrow (plastic ABS pipe approximately 17 cm long with a 6 cm diameter opening). Animals were fed 60 g of mixed seeds and two commercial rodent chow blocks once a week and one piece of Romaine lettuce three times a week as a water source.

#### **V.4.3. Procedure**

The burrow-pipe was removed from each individual's colony cage in the afternoon of each sampling day (1300 to 1700 hours). The material was sifted to separate the food from the sand, and the former was weighed to determine the amount of food in grams the

individual stored in the burrow-pipe. The seeds and sand were returned to the burrow-pipe, which was replaced in the colony cage.

#### **V.4.4. Data collection**

The only measure collected was the grams larder hoarded on each day of sampling.

#### **V.4.5. Results**

There was no overall difference in the amount of larder hoarding of animals that were trapped as adults and those trapped as infants ( $F(1,26) = 0.09$ , n.s.); all subsequent data pools the data from the two subpopulations. The average amount of larder hoarding across days of the experiment was similar across subjects ( $N = 28$ , each sampled eight times for a total of 224 data points, mean (SD) = 15.78 (21.52)); the minimum average value ( $N = 28$ , mean (SD) = 8.32 (8.85)) took place on the last day due to the timing of cage changing by animal care technicians. This contributed to a significant effect of day ( $F(7,182) = 3.40$ ,  $p = .002$ ). There was individual stability in the amount of larder hoarding, with the data from each individual correlated across all eight days of the experiment, except for the last day (when the cages were changed) ( $r > .472$ ,  $p < .05$ ). The average amount of larder hoarding was higher for males ( $N = 13$ , mean (SD) = 22.61 (28.63)) than for females ( $N = 15$ , mean (SD) = 9.34 (2.71)), but this difference was not statistically significant ( $F(1,26) = 2.59$ ,  $p = .12$ ).

There was no blood collected at the time that these hoarding data were taken. However, since there are stable individual differences in the levels of larder hoarding and there may be stable individual differences in the levels of circulating glucocorticoids,

then there could be a correlation between the two measures even using samples taken from different times. I compared the data from the two baseline blood samples to the amount of larder hoarding. There was no overall correlation between average grams of food larder hoarded across all days and average corticosterone level across the two baseline samples ( $r = .20$ , n.s.), but since the two baseline corticosterone samples were not correlated with each other ( $r = .29$ , n.s.), individual correlations with each sample were run. While the corticosterone levels in the first sample did not correlate with the grams larder hoarded ( $r = .10$ , n.s.), the corticosterone levels in the second sample did ( $r = .55$ ,  $p = .015$ ).

#### **V.4.6. Discussion**

There is great individual variability in the use of the home burrow to larder hoard food in the home cage. Similar to the sex difference in corticosterone data, males larder hoarded more than females. Males also weighed more than females, but weight alone does not seem to explain the sex difference in larder hoarding. Baseline corticosterone samples 1 and 2 were not correlated with each other, suggesting that there were some differences in the stress levels in the animals at the time of collection or that the individual variation is greater than the between-individual variation.

Although sample 1 was not related to the individual differences in larder hoarding, sample 2 was; higher levels of stress hormone were associated with more larder hoarding in the home burrow. This difference between sample 1 and 2 is not likely to be due to the fact that the second blood sample was taken closer in time to the hoarding data

than the first blood sample; both blood samples were taken over two months prior to the hoarding data so neither or them are precise reflections of the state of the animal at the time of caching.

This behavioral measure is reliable, stable and is an easy way to determine individual differences in cache strategy. The measure needs to be replicated with blood data that is taken at the time of the larder measurement in order to confirm any relationship between stress and hoarding.

## **V.5. Experiment 8: 0, 1, 2 days food deprivation**

This experiment was described in detail in the previous chapter. The hormone data from the food-deprivation experiments are presented here along with a series of methodological verification experiments so that the strengths and weaknesses of the blood-collection procedure can be directly compared across experiments.

### **V.5.1. Study animals**

The experiment was conducted at the University of California at Berkeley in the fall of 2000. I examined 23 wild-caught Merriam's kangaroo rats (10 females and 13 males). Kangaroo rats were trapped in Palm Desert, CA in January of 2000, and Palm Springs CA, in April of 2000. They were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

### **V.5.2. Apparatus**

Cache trials were conducted in the small arenas with wire lid tops as described in Chapter III. Six animals were run at a time. The room was lit with two 25 watt red bulbs. There were numerous extra-apparatus cues in visible in the room.

### **V.5.3. Procedure**

After each 24 h cache trial (described in section IV.2.3.), the anesthesia and blood collection supplies were brought into the colony room adjacent to the procedure room. At approximately 5 pm, each subject was carefully removed from the cache arena while still inside the home burrow, placed in the home cage, and the home cage was placed in the colony room. One at a time, an induction chamber filled with isoflurane was brought into the colony room, an animal was placed into the chamber and brought into the procedure room. Blood collection proceeded as described in the general methods for hormone analysis (section V.2.). After each subject was sampled, it was left in the procedure room to minimize disturbance to the colony room and the next subject was retrieved from the room in the induction chamber. After all six subjects were sampled, the blood was centrifuged and the plasma was stored, the subjects were all returned to the colony room and given their ad libitum supply of food.

### **V.5.4. Data collection**

The time that the experiment room was first entered on the day of sampling, the amount of time it took for each subject to be anesthetized, and the handling time for each subject was recorded. The weight and reproductive status was also taken of each subject at the time of blood collection.

### **V.5.5. Results**

Individual differences in corticosterone levels were stable across the food-deprivation conditions (0, 1, 2 days;  $R^2 > .29$ ,  $p < .01$ ; Table V.1.). Males had higher levels of corticosterone than females ( $F(1,21) = 5.325$ ,  $p = .03$ ). Corticosterone levels did not change with the amount of deprivation ( $F(2,42) < .512$ , n.s.). Overall, corticosterone levels were not related to cache strategy ( $r < .318$ , n.s.).

Across all subjects in the experiment ( $N = 69$ ), there was no significant relationship between the order that the subjects were run and the corticosterone level ( $r = -0.21$ ,  $p = .09$ ), the direction of the relationship was negative with lower corticosterone levels for subjects run later. Based on the descriptive data (see Table V.1.), it seemed that the first subject of each day had a higher value than all other subjects, so post-hoc tests were run to test this assumption. There was no difference among the subjects taken second through sixth ( $F(4,51) = .231$ , n.s.), but with a binary code for order as the independent variable (1 = first subject that day, 0 = all other subjects) and corticosterone level as the dependent variable, the first subjects of the day had significantly higher levels of corticosterone ( $N = 13$ , mean (SD) = 29.56 (16.30)) than all other subjects ( $N = 56$ , mean (SD) = 21.76 (9.20)) ( $F(1,67) = 5.49$ ,  $p = .02$ ).

### **V.5.6. Discussion**

There may be some something wrong with the hormone assay, and there may be a handling stress confound in the collection procedure. There was no overall order effect or effect of blood collection time, but the first subjects run on each day had higher

corticosterone levels than the other subjects, suggesting that the animals were stressed by the initial disruption of the animals in the experiment room and the process of moving them into the colony room. If they were stressed by this move, however, the stress seemed to decrease after a few minutes since all remaining subjects had equivalent levels of the stress hormone.

Hormone levels did not increase across levels of deprivation. There are three obvious reasons that may have precluded an effect. Kangaroo rats may have a shifted range of corticosterone with low levels near 0 and high levels under 100 ng/ml as such, the levels that have been recorded in all previous samples may have reflected a ceiling effect rather than floor effects of stress. If this were the case, then stress from the handling and collection procedure is overshadowing changes from the manipulation. Another potential problem is that two days of food deprivation is not enough to stress this species, which is adapted to an environment with an unpredictable supply of food. Furthermore, cortisol, not corticosterone may be the primary stress hormone in Merriam's kangaroo rats. Most importantly, the blood samples were taken after the cache trials. As such, the subjects may not still have been stressed from the food deprivation as they all ate seeds during the 24 h cache trial. Data from chickens suggests that even seeing food after a period of food deprivation is enough to ameliorate the stress (Harvey et al., 1983). Therefore, an additional experiment was run to correct these problems. In Experiment 9, there were short and long-term food deprivation conditions, blood was collected at the time of deprivation, after each day of deprivation, and before the cache

trial, assays for corticosterone and cortisol were run and the weight of the animals was monitored carefully each day of the experiment.

## **V.6. Experiment 9: Short- versus long-term food deprivation**

### **V.6.1. Study animals**

The experiment was conducted at the University of California at Berkeley in April of 2001. I examined 12 wild-caught Merriam's kangaroo rats (6 females and 6 males).

Kangaroo rats were trapped in Palm Desert, CA in January of 2000, and Palm Springs CA, in April of 2000. They were maintained in captivity at the University of California at Berkeley on a 12:12 h light cycle with lights on at 0400 h.

### **V.6.2. Apparatus**

Cache trials were conducted in the small arenas with wire lid tops as described in Chapter III. Six animals were run at a time. The room was lit with two 25 watt red bulbs. There were numerous extra-apparatus cues in visible in the room.

### **V.6.3. Procedure**

This experiment had a mixed design as described with the cache data in Chapter IV (see Fig IV.8.). There was a between-subjects design for the short-term deprivation replication of the previous experiment (1 versus 2 days deprivation); Group 1 was deprived and given a cache trial one day later while Group 2 was deprived and given a cache trial two days later. There was a within-subjects design to compare short-term with long-term

deprivation (<2 days or >7 days). Both groups were given a cache trial approximately 1 week after their short-term deprivation experiment. Therefore, each subject had two 24 h cache trials in the same small multiple-subject arena with 100, shelled sunflower seeds and 2 g Romaine lettuce each time.

Each subject was monitored for each day that it was on food deprivation. The weight and blood of the animal was taken before food deprivation started, and each day that the animal was on food deprivation. For the day that each animal was scheduled for a cache trial, the blood was taken before the cache trial to eliminate the effects of eating and then the subjects were placed into the cache arenas. One at a time, the subjects were brought from the colony room into the adjacent procedure room in their home cage. The subject was transferred from the home cage into the induction chamber that was connected to the isoflurane machine. The blood was collected as described in the “general methods for hormone analysis” (section V.2.). After the sample was collected for each individual, the animal was returned to its home cage. After all samples for the day were collected, the subjects were placed into the cache trial arenas and left overnight.

The decision to collect blood samples from the long-term condition was post-hoc, and the procedures did not match those of the short-term condition exactly. Only four subjects from each group were sampled, and blood was collected in a different room. Subjects were brought from the colony room to a procedure room down the hall en masse on a cart. Then blood was sampled serially in the procedure room as described above.

#### **V.6.4. Results**

For the between-subjects comparison of 1 to 2 days deprivation, there was not a difference in stress hormones for corticosterone ( $F(1,7) = 0.23$ , n.s.) or cortisol ( $F(1,7) = 0.001$ , n.s.). One day of food deprivation yielded a mean of 33.11 ng/ml for corticosterone ( $SD = 4.92$ ) and 31.12 ng/ml for cortisol ( $SD = 7.57$ ); two days of deprivation yielded a mean of 14.77 ng/ml for corticosterone ( $SD = 10.17$ ) and 15.02 ng/ml for cortisol ( $SD = 9.64$ ). The corticosterone data was not correlated with the cortisol data ( $r = 0.32$ , n.s.), but after the corticosterone assay was run, there was only enough plasma in nine of the twelve samples for cortisol analysis. There was also no significant difference in weight between the subjects that had 1 or 2 days deprivation ( $N = 6$  in each). There was an average difference from 39.45 ( $SD = 2.91$ ) in the 1 day animals to 37.98 ( $SD = 5.25$ ) in two day animals, but this difference was not significant ( $F(1,10) = 0.36$ , n.s.).

Since Group 2 animals were sampled on the first and second day of their deprivation, there was also the possibility to replicate the effect with a within-subjects comparison that would eliminate the problem of individual variability (albeit with only 6 subjects). With a repeated-measures ANOVA on the six subjects in Group 2, there was virtually no difference between the level of corticosterone after 1 day of deprivation (mean ( $SD$ ) = 32.79 (13.54)) and the level after 2 days of deprivation (mean ( $SD$ ) = 33.00 (7.37)) ( $F(1,5) = 0.003$ , n.s.), even though the loss in weight for the within-subjects comparison from 1 to 2 days (mean percent loss ( $SD$ ) = 3.13% (0.73)) was significant ( $F(1,5) = 103.68$ ,  $p < .001$ ). Therefore, there are three different replications from different

experiments or analyses that do not find a difference in stress between one and two days of food deprivation.

There was no correlation between the order subjects were sampled within their deprivation group (ordered 1 through 6 for both deprivation groups) for corticosterone (N = 12 (two in each order),  $r = 0.42$ , n.s.) or cortisol (N = 8,  $r = 0.19$ , n.s.). There was also no correlation between the amount of time it took to sample each individual and the level of corticosterone (N = 12,  $r = -0.025$ , n.s.) or cortisol (N = 8,  $r = -0.016$ , n.s.).

Because the 1 and 2 day deprivation groups were tested at different times of day (approximately 1930 h and 1630 h, respectively) the time of day that the blood was collected was also considered a factor. There was no correlation with the time of day that the blood was sampled and the corticosterone levels (N = 12,  $r = 0.04$ , n.s.) or the cortisol levels (N = 9,  $r = 0.18$ , n.s.). There was also not a categorical effect of the blood collection being earlier versus later on corticosterone levels ( $F(1,10) = 0.001$ , n.s.) or cortisol levels ( $F(1,6) = 0.17$ , n.s.).

For the within-subjects comparison, increasing food deprivation significantly *decreased* levels of circulating corticosterone (from no deprivation to 1 day to 1 week:  $F(2,12) = 4.83$ ,  $p = .029$ ; Table V.2.). Moreover, depriving subjects for one day only significantly *decreased* levels of circulating corticosterone (from no deprivation to 1 day:  $F(1,12) = 6.099$ ,  $p = .03$ ). There was no interaction of this effect with gender ( $F(2,12) = 0.14$ , n.s.). Combining across all hormone data in a between-subjects fashion (ignoring the fact that each individual was sampled two or three times), the means values for both

corticosterone and cortisol decreased with the length of deprivation (see Tables V.1. and V.2.).

With data from only four subjects that had weight data from all three days of deprivation (none, one day, long-term), there was a significant decrease in the weight of the animals ( $F(2,6) = 283.98, p < .001$ ). The average percent loss from 0 days deprivation to 1 day deprivation was 6.30% ( $N = 6, SD = 1.54$ ), the loss from 1 day to over 1 week was 17.11% ( $N = 8, SD = 1.78$ ).

Because the blood collection procedure for the long-term deprivation subjects was different from the others, an additional set of correlations was performed to determine if there were any order effects or effects from handling. With data from the only eight long-term deprivation subjects, there was no correlation between the order that the animals were sampled and the level of corticosterone ( $r = 0.43, n.s.$ ), but there was a trend for the amount of time it took to sample each individual and the level of corticosterone ( $r = 0.69, p = .08$ ). The corticosterone data seemed increased for samples that took longer than two minutes ( $N = 4, \text{mean (SD)} = 32.44 (3.05)$ ), compared to those collected in less than two minutes ( $N = 4, \text{mean (SD)} = 21.08 (3.75)$ ). Using a one-way ANOVA (with less than or more than two minutes as the independent variable), the level of corticosterone in the “under two minutes” samples was significantly lower than in the “over two minutes” samples ( $F(1,6) = 22.09, p = .003$ ).

#### **V.6.5. Discussion**

Replicating the null effect from Experiment 8, Experiment 9 did not produce a significant change in the levels of corticosterone from 1 to 2 days using the between-subjects design. One could again suggest that 1 to 2 days of deprivation was not enough to stress the animals, or for them to lose a significant amount of weight. Indeed, there was not a significant difference in weight between animals that had 1 versus 2 days of deprivation.

However, using the within-subjects data from Group 2 subjects (that were weighed and sampled 1 and 2 days after deprivation), the loss in weight from 1 to 2 days was statistically significant, yet there was absolutely no decrease in their levels of corticosterone from one day to the next. Therefore, a lack of weight loss would not explain the null result, except that a statistically significant loss in weight may be different from a functionally important loss.

Even though subjects only lost an average of three percent of their body weight from day 1 to 2, this was already after a six percent average loss from 0 to 1 day of deprivation, therefore, by the second day of deprivation, subjects had lost an average of nine percent of their body weight. However, when the day 2 weights are compared to the weight of the animals when they were trapped in the field, the weight after deprivation is still actually seven percent higher on average than the weight of the animals when they were trapped. The results are the same even after comparing the weights of the animals post-quarantine. Therefore, although the weight data suggest that the animals should have been stressed, they may not have been unduly stressed since they were still, on average, above their weight from the field.

Weight may not be a linear function, and may be better modeled as a catastrophic surface where the effect of the weight change depends on what the weight was locally, more than globally. If this is the case, then the local, sudden loss in weight may have still been stressful to the animals, even if there were no measured changes in glucocorticoids.

One might also suggest that the lack of effect in this experiment was due to the fact that the data were compared between individuals since the individual variation may overshadow the relative changes in stress within an individual from 1 to 2 days of deprivation. However, there was also no effect in Experiment 8 nor when comparing the data of Group 2 subject in Experiment 9 (both of which were within-subjects of 1 to 2 days). This fact makes it unlikely that a variance problem explains the null result.

There were also no significant relationships between the levels of glucocorticoids and factors introduced by the blood collection procedure, suggesting that the lack of an effect of the manipulation is not due to a ceiling effect of stress from the procedure per se. However, the procedure may still have caused a ceiling effect if the mere onset of the procedure (i.e. entering the room the first time) was stressful enough to cause an increase in even the first subjects, in the time it took to withdraw their blood. If this were the case then the animals in all of the data collected thus far may have had uniformly high levels of glucocorticoids compared to their usual baseline, and they would have very low levels of glucocorticoids compared to rats and mice.

Contrary to the expected increase in stress from the long-term food deprivation, long-term food deprivation actually appeared to cause a *decrease* in stress; values

decreased with the length of deprivation from none to one day to over one week. It is highly unlikely that the animals were not stressed by the long-term condition because one animal died from the stress one day before the cache trial. Comparing the weight of the animals after the long-term deprivation to their entry weights, of four animals that had weights for both conditions, there is an average loss of 7.37% (SD = 13.67); one of the four animals was still at a higher weight than in the field (but it should be noted that field weight data is not as reliable as in the laboratory because the portable hanging scale used in the field is less precise and more variable).

It seems unlikely that the lack of an increase in the glucocorticoids of food-deprived animals is due to the lack of stress, for the reasons given above. It seems more likely that either this is a very sensitive animal that has a very low baseline level of stress that is not being reflected in any of our measures due to handling stress, or that these animals have a suppressed glucocorticoid response to stress due to their adaptation to desert conditions, and possibly their breeding condition (this later factor is discussed in more detail below).

#### **V.6.6. General discussion**

The two food-deprivation experiments did not yield increases in glucocorticoids despite replications with three different subpopulations. Animals did lose weight from the deprivation and did change their hoarding (see Chapter IV), but they did not lose a significant amount of weight when compared to their original weight in the field. There was great individual variability in the amount of weight change from trapping, to

postquarantine, to their experimental weight, with a substantial number of increases and decreases. Therefore, the individual animals may have different long-term strategies for balancing the use of body fat and stored or foraged food for survival. If there is in fact such variability, then greater numbers of subjects would be needed and a more detailed analysis of metabolic factors such as stored fat, body temperature, and foraging time would be necessary to shed light on the role of stress hormones on hoarding.

It is also unclear if the hormone assay kit is actually valid for use with kangaroo rats. If the assay is not binding to the hormone sufficiently, then consistent and unrepresentative low values would result, such as I have gotten in these experiments. In addition, kangaroo rats may have low levels of circulating corticosterone, in which case, the values generated thus far would actually be ceiling effects of stress from the blood collection procedure.

In order to try to tease apart some of these issues, a series of follow-up blood collection procedures were run, based on the findings of the literature.

## **V.7. Experiment 10: Procedures to validate the hormone collection and assay procedures**

### **V.7.1. Restraint stress**

**Methods** Subjects were brought into the procedure room en masse and given at least thirty minutes to acclimate to the new location. One at a time, the subjects were anesthetized within 1 minute, and blood was drawn within 3 minutes (?) to establish a baseline measure. After this, the subject was warmed until it began to wake up, and placed into a plexiglass mouse restraining apparatus that allowed the subject to wiggle, but not make overt movements. All subjects appeared by visual inspection to be stressed by the procedure, either wriggling excessively or being completely frozen.

Baseline data was also taken in the same manner as described above (without the restraint) at different times of day, to determine if there was a circadian rhythm to the glucocorticoids release, and if this rhythm could have overshadowed any normal increases due to stress.

**Results** There were no significant differences in circulating levels of corticosterone from before to after restraint stress ( $F(1,4) = 3.31, p = .14$ ). The experiment had low power, but two of the six subjects had even lower corticosterone values after the restraint. There was a significant difference in the levels of corticosterone throughout the day. ( $F(3,19) = 3.53, p = .03$ ), with values peaking in the afternoon (2pm:  $N = 6, M = 36.67, SD = 6.04$ ; 5pm:  $N = 6, M = 32.83, SD = 4.58$ ) and lower in the morning and evening (11am:  $N = 5, M = 28.45, SD = 2.13$ ; 9pm:  $N = 6, M = 27.76, SD = 5.69$ ).

**Discussion** The data from the restraint manipulation and the time of day analysis further suggest that the hormone assay is not accurate, or that these are unusual subjects.

There was not a consistent increase in stress after restraint, the most common stressor in the animal literature. One could suggest that restraint in a small plastic tube is not stressful for a burrow-living animal. The animals did appear to be stressed, and either attempted escape or lay frozen for the duration. Thus, it seems more likely that the baseline values were elevated, precluding a significant increase. The fact that the baseline levels in the time of day analysis were high at the time that the experiments are run suggests that maybe the endogenous effects of circadian rhythm suppressed the response to exogenous factors at this time of day. There is precedence for a lack of effect during the peak time of day in a hamster experiment, even though the effect of the manipulation was evident at other times of day (Blum et al., 2001).

#### **V.7.2. Cortisol versus corticosterone**

There is variability across rodent species in the relative importance of cortisol versus corticosterone. In rats and mice, corticosterone is the primary glucocorticoid (Muridae/Murinae, Hawkins et al., 1975; Walker et al., 1992). In primates, cortisol is the primary GC, leading researchers and medical science to make the generalization that non-primates use corticosterone and primates use cortisol. This, however, is not the case, as there is great variability across rodent species.

In caviomorph rodents cortisol, not corticosterone, is the major glucocorticoid. This includes guinea pigs (Cavia porcellus, Dalle & Delost, 1974), degus (Octodon degus, Kenagy et al., 1999) and yellow-pine chipmunks (Tamias amoenus, Kenagy & Place, 2000). Noncaviomorph squirrels (Spermophilus saturatus, Boswell et al., 1994;

Marmota, Kastner et al., 1977; Citellus citellus, Shivatcheva et al., 1988) and gerbils (Psammomys obesus, Amirat et al., 1980) also follow this trend. In some cases, both GCs are present and can be assayed, but one is an order of magnitude or so higher than the other (e.g. Kenagy & Place, 2000). In degus (Octodon degus), the levels of corticosterone are low enough to be caused by cross-reactivity of cortisol with the corticosterone assay (Kenagy et al., 1999). In the golden hamster (Mesocricetus auratus), both GCs are present in comparable levels (Ronchi et al., 1998). Absolute levels of GCs are also considered in the literature. High absolute levels are associated with low binding affinity (Keightly & Fuller, 1995; Kenagy et al., 1999).

**Results**        The results from the cortisol assay were virtually identical to those from the corticosterone assay ( $r = .406$ ,  $p = .001$ ). Whereas the correlation of weight with corticosterone was not significant ( $r = .157$ ,  $p = .09$ ), the correlation of weight with cortisol was significant ( $r = .484$ ,  $p = .002$ ). As with corticosterone, there was not a significant correlation between the time required to withdraw the blood and cortisol values ( $r < -.234$ ,  $p > .05$ ), but again, both  $r$  values were unexpectedly negative. Again, this measure does not include all of the time that the experimenter was in the room, only the time required to extract the blood from a single subject from the time that anesthesia starts.

**Discussion**    These data suggest that either the hormone assay kits are not specific enough to detect glucocorticoids in kangaroo rats, or that kangaroo rats have unusually low levels of glucocorticoids, and these data still reflect ceiling effects from the blood

collection procedure, rather than floor effects from the lack of stress. This latter hypothesis is possible since, in the latter manipulation checks, all subjects were brought into the lab on a cart, which may have been disruptive enough to cause stress-like increases in the baseline measures.

It is unlikely from these data that kangaroo rats disproportionately use cortisol or corticosterone or that the lack of effects in the former studies was due to the assay of the wrong major GC. The cortisol values were slightly lower than those of corticosterone, but this may be an artifact from the fact that the cortisol assay was done on the remains of the blood plasma after the corticosterone assay. Thus, some samples had too little plasma remaining for the cortisol assay, which may have yielded spuriously low values for cases that were not obvious.

## **V.8. General discussion**

There are many possible reasons for the lack of conclusive findings from these hormone experiments. Some of the possible reasons have been tested (including that the animals were not stressed enough and that they use cortisol instead of corticosterone), and still there is uncertainty as to whether the hormone assay even works. There most likely reason for the null results after all three major sets of experiments is still that the Merriam's kangaroo rats have extremely low levels of glucocorticoids that react quickly to stress, such that the apparent lack of stress actually reflects a uniformly high level of

stress across conditions from the procedure itself. There are still two factors that may have contributed these data that will be discussed now.

Corticosterone can be floating in the blood or bound to corticosteroid-binding globulins (CBG's). The bound particles cannot cross the membrane to affect the cell. A standard RIA measures only free, unbound corticosterone. Preceding the assay with a dichloromethane extraction procedure separates the bound corticosterone from the CBGs, yielding a measure of free and bound corticosterone and thus a higher overall level of corticosterone. If there had been an extraction procedure, the values obtained would have been higher, which is sometimes necessary for a statistically powerful effect of a stress manipulation.

Another possible reason for these unusual stress hormone results is the extreme environmental conditions to which this species is adapted (i.e. the desert). There is evidence that in species from extreme environments, the stress response can be suppressed or eliminated to prevent the consequences of high GCs on breeding. Birds that live in temperate zones terminate breeding attempts in response to inclement weather, correlated with a high level and high responsiveness of corticosterone (e.g. song sparrows (Melospiza melodia), Wingfield, 1985a; Wingfield, 1985b; white-crowned sparrows (Zonotrichia leucophrys pugetensis), Wingfield et al., 1983). Desert-living birds also suppress their response to stress during summer breeding when the water supply is low (Wingfield et al., 1992). But, across multiple bird species sampled from different locations in Alaska (where sudden storms often occur during the breeding season), only

some showed a suppressed corticosterone response to capture and restraint stress (Astheimer et al., 1995; Wingfield, 1994; Wingfield et al., 1995; Wingfield et al., 1994b). In one study, the suppressed response effect was most correlated with the degree of parental care characteristic of the species (Wingfield et al., 1995). In another study, the reduced responsiveness to capture, handling and restraint seemed to occur in species that lived at higher altitudes, where the temperature is colder, and where animals develop greater fat reserves that would suppress the response to stress (Wingfield et al., 1994a).

If GC responsiveness really is suppressed when it is more adaptive to continue with a breeding attempt despite conditions, then depending the particulars of the species, there should be sex differences in this behavior. In Gambel's white crowned sparrows (Zonotrichia leucophrys gambelii), females show a much lower response to capture stress than males (Wingfield et al., 1982; (Wingfield, 1988). During the breeding season only, males appear to have an increased responsiveness to stress due to a decrease in negative feedback at the HPA axis (Astheimer, Buttemer, & Wingfield, 1994). King penguins have low corticosterone levels during incubation even though they are voluntarily fasting, but this effect goes away when body fat drops below a certain level (Le Ninan et al., 1988).

When molting, it would be adaptive to decrease GC responsiveness to allow for maximal anabolic activity (i.e. feather growth), which is normally inhibited by glucocorticoids (Astheimer et al., 1995). Therefore, this effect should occur across sexes. Male and female Lapland longspurs (Calcarius lapponicus), Gambel's white-crowned

sparrows (Zonotrichia leucophrys gambelii), and song sparrows (M. melodia) all suppress their corticosterone response to stress during molting (Astheimer et al., 1994; Astheimer et al., 1995; Astheimer, unpublished, respectively).

Oftentimes, the baseline levels of animals in high and low stress situations do not differ, but the animals in the high stress situation have an increased response (faster and larger) to restraint stress (Wingfield, 1994). Low food levels increase the corticosterone response to stress in Black-legged Kittiwake chicks (Rissa tridactyla); this response is exacerbated when animals are fed food that is low in fat relative to protein. The more fat reserves, the lower the corticosterone response to stress (Kitaysky et al., 1999a; Kitaysky et al., 1999b).

The above examples from extreme environment animals are all from avian species, therefore the validity of comparing these results to mammals and then to kangaroo rats should be questioned. However, the neural distribution of corticosterone receptors in birds is homologous to that of mammals using autoradiography (Kovacs et al., 1989; Kovacs & Peczely, 1986) and the corticosterone effects of body mass and reproductive state have been replicated in other vertebrate groups. Frogs (Rana esculenta) have a reduced elevation in corticosterone in response to capture stress during the breeding season compared to other times of year (Paolucci et al., 1990). Desert lizards (Amphibolurus) show an inverse relationship between body mass and corticosterone (Bradshaw, 1986). Female whiptail lizards (Cnemidophorus uniparens) have lower baseline levels of corticosterone and reduced responsiveness to capture and cold stress

and before ovariectomy (Grassman & Crews, 1989). In laboratory rats, the basal level of corticosterone is elevated during lactation and the response to stress is suppressed (Lightman, 1992; Lightman & Young, 1989).

Based on these studies, it seems unlikely that kangaroo rats would be in a state of stress suppression since most of the above literature refers to animals that are breeding and molting. The subjects in these studies were not given access to potential mates, but they may have been reproductively active since they were fed copious amounts of lettuce as part of their per diem allotment of food. Since opportunistic breeding in this species is initiated by the presence of leafy green plants, the abundance of lettuce in their ad libitum diet may have kept these animals in a state of reproductive readiness. However, most of the above data is on animals that have already mated, not animals simply in the breeding season. In the future, these questions may be answered by collecting vigorously controlled baseline samples that are highly unlikely to be contaminated from procedure stress, and by collecting more blood from each individual so that assays can be run in parallel on different hormones, with and without an extraction procedure.

**Table V.1.**

Descriptive Statistics for food deprivation condition and the level of corticosterone (ng/ml) from Food Deprivation Experiment 8.

Gender	Days Deprivation	Mean	Std. Deviation	N
Males	0	27.37	14.05	13
	1	28.17	11.15	13
	2	25.44	12.33	13
	Total	27.00	12.29	39
Females	0	20.03	5.56	10
	1	17.24	3.94	10
	2	17.72	10.61	10
	Total	18.33	7.14	33

**Table V.2.**

Descriptive Statistics for food deprivation condition and the level of glucocorticoids (ng/ml) for Food Deprivation Experiment 9.

Assay	Days Deprivation	Mean	Std. Deviation	N
Corticosterone (ng/ml)	0	36.36	11.37	17
	1	32.94	10.07	11
	2	31.12	7.57	4
	7	29.59	.	1
	Total	34.38	10.32	33
Cortisol (ng/ml)	0	24.54	11.59	17
	1	17.08	12.57	11
	2	15.02	10.47	4
	7	4.18	.	1
	Total	20.28	12.31	33

# Chapter VI

## General Discussion

Studying cache strategy in Merriam's kangaroo rats utilizes natural, complex behaviors to investigate the proximate mechanisms of behavior, and thus offers a favorable animal model for studying the interaction between economic decision-making processes and memory. I hypothesized that variation in cache strategy is mediated by arousal, implicating catecholamines, glucocorticoids, benzodiazepines or some combination of the above since they all influence arousal and memory. I further hypothesized that such variation in cache strategy adaptively complements changes in memory performance that accompany arousal. If cache strategy and memory performance follow the same inverted-U shaped curve of arousal, animals should scatterhoard when memory performance levels are high. The levels of circulating glucocorticoids were measured to determine the contribution of this stress hormone towards food-storing decisions and memory for cache locations in Merriam's kangaroo rats.

The results across experiments yield some important generalizations. Animals increase the use of caching overall across days of an experiment, and with increases in food deprivation. This supports both a optimization model of caching where food is carried farther from the source over time, and an anticipation model of caching where

there must be cognitive and emotional anticipation of a food shortage in order to engage in caching (implicating the dopaminergic, frontal cortex system).

Consistent with my prior experiments, actual pilferage and the presence of the competitor are required to elicit a change in cache strategy or cache location. Thus far, presence alone, pilferage alone, and pilferage with scent cues of a competitor are not enough in isolation to elicit a change. These results should not be generalized to natural conditions, since there are many reasons why the lab circumstances do not mimic the conditions in the field. In the field, it is likely that the presence and number of competitors are always correlated with the amount of pilferage. Most importantly, pilferage in the field has a much more profound effect on the animals because they must travel large distances across their home ranges to locate caches and make compensatory foraging trips when caches are pilfered.

Future experiments should more closely mimic natural conditions where animals must travel a distance to “forage” for the seeds, pilferage is defined as seed loss rather than seed movement, and new cache preferences are assayed from new seeds that the subject has to forage.

Based on the series of hormone experiments and follow-up analyses, it seems that the original baseline data may have been misleading. The original values were consistent with baseline values in rats and mice, but these values did not change significantly across conditions that should have been stressful. This suggests that kangaroo rats have unusually low levels of circulating glucocorticoids, and that they have responsive systems

with high binding affinity such that low absolute levels of GCs are able to adaptively change the state of the animal.

It is still possible that the lack of stress response in these individuals is because of the species' status as an individual from an extreme environment (discussed in the previous chapter). This seems unlikely since most of the literature on extreme environment animals refers to the need to suppress stress during breeding and molting. These individuals were not breeding, but they may have been reproductively active since they were fed copious amounts of lettuce as part of their per diem allotment of food. This lettuce may have signaled reproduction and caused a subsequent suppression of stress hormones.

Future research is aimed at, 1) replicating the long-term food deprivation effects on caching to eliminate the order effect, and 2) obtaining larger quantities of blood so that a more detailed assay analysis can be done. This latter effort will try to assay the same samples with and without an extraction procedure, and with corticosterone and cortisol. In addition, samples will be taken before and after restraint and with or without food deprivation. A replication of the time of day analysis will also be done. Because these procedures are terminal, the animal's whole volume of blood can be collected within two minutes, avoiding the increase in GCs from handling stress in the blood sample. In addition, a modified version of the remote sampling technique of Reburn & Wynne-Edwards (2000) will be attempted so that baseline samples for a restraint stress protocol

can be taken with almost no disturbance to the animals before the blood is collected, and with all subjects in the room sampled at the same time.

Future studies will be aimed at testing the interaction of stressful conditions with memory in order to establish if in fact scatter hoarding takes place during peak memory performance and vice versa. Once a way is developed of taking repeated blood samples from the same individual without handling stress, myriad experiments can be done that would greatly contribute to our understanding of behavioral neuroendocrinology. The addition of pharmacological manipulations would also elucidate the proximate mechanisms in natural, behaviorally complex experiments. Merriam's kangaroo rats provide a unique species to study complex interactions between decision making, stress and metabolism in the laboratory. The effort to determine the physiological parameters of stress hormones in this species and the techniques necessary to assay them will come with a great reward. This information will allow us to understand how this unique species is adapted to its environment and what role stress hormones play in the proximate mediation of hoarding behavior. Moreover, this species can be used as a model for humans because the interface between stress, decision making and memory can be studied in the laboratory with a complex, natural paradigm.

Hoarding is a general phenomenon that exists across species, and in many contexts. Humans typically hoard valuable resources, especially food, money, and information, and there is great individual variation in the extent or type of hoarding. Many patient populations are characterized by their aberrant hoarding behavior.

Individuals with Obsessive Compulsive Disorder (OCD), tic disorders, schizophrenia, dementia, and frontal lobe damage all exhibit pathological hoarding (Damecour & Charron, 1998). A common underlying factor in these patients groups is the lack of insight and control over this behavior, implicating the decision-making complex of the ventromedial prefrontal cortex (Bechara et al., 2000) and the frontal dopamine system (Damecour & Charron, 1998). Moreover, larder hoarding of non-limited items is known to occur in people that experienced stressful life history events such as war or economic depression. These behaviors last long after the supply crisis and appear to reflect permanent changes in hoarding circuitry as the result of extreme stress, possibly implicating damage from a chronic overload of glucocorticoids as is seen in individuals with post-traumatic stress disorder (PTSD) (e.g., see Koenen et al., 2000).

There is rich evidence to suggest that hoarding is a general, adaptive phenomenon that has a biological basis and that this proximate basis can be disrupted by brain damage. The investigation of hoarding behavior in animals such as the Merriam's kangaroo rat, at behavioral and biological levels, can be applied to the understanding of such disorders. This research can also be used to understand between- and within-individual differences since hoarding behavior should be sensitive to past, current, and predicted future levels of available resources. This ubiquitous phenomenon has great adaptive benefits for any animal that experiences fluctuations in the supply of resources that are necessary for survival.

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