

SPATIAL MEMORY RECALL IN THE GIANT PANDA
(AILUROPODA MELANOLEUCA)

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SPATIAL MEMORY RECALL IN THE GIANT PANDA
(AILUROPODA MELANOLEUCA)

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SUMMARY

The giant panda (*Ailuropoda melanoleuca*) is an endangered species and many efforts are being made to ensure its survival, including numerous research studies. However, there has been little investigation of spatial memory in the giant panda. Spatial memory is an important mechanism for survival in the wild, allowing an animal to find and remember the location of food, mates, den sites and avoid predators. Memory assessment in non-human species typically involves the use of recognition, as opposed to recall tasks. The current study tested spatial memory recall in 1.1 giant pandas using a delayed response memory task. The design required a delayed response to a previously lighted location, with varying lengths of delay between the observation phase and the test phase. The male subject reached criterion at 2-, 3-, 4-, 5-, 6-, and 10-second delays. The female subject reached criterion at 2-, 3-, 4-, 5-, 6-, 10-, and 15-second delays. The results support the hypothesis that giant pandas have working memory recall ability for spatial location.

CHAPTER 1

INTRODUCTION

The giant panda (*Ailuropoda melanoleuca*) has stimulated a great deal of research in zoological institutions because of its unique biological characteristics, difficulties in captive breeding, and conservation efforts (Lindburg & Baragona, 2004). The captive environment provides a unique opportunity to investigate sensory and cognitive abilities which could not be studied in the wild, but may be relevant to the conservation of giant pandas. Survival in the wild depends on an individual's ability to navigate its environment to locate resources such as food, potential mating partners, and avoid predators (Vauclair, 1996). Therefore, a lot of research has focused on giant panda sensory capabilities, including olfactory communication and vision (Swaigood, Lindburg, White, Zhang, & Zhou, 2004; Kelling et al., 2006). Yet, there has been little investigation of spatial memory in the giant panda, which may be a critical component of efforts such as foraging for bamboo (Tarou, Snyder & Maple, 2004).

More research is needed to investigate giant panda cognition and memory. Furthermore, the majority of memory research in non-human species has focused on recognition, rather than recall memory (Washburn, Gullledge & Martin, 2003). Cognitive tasks may also serve as enrichment for animals in captivity, providing novel stimuli that enhance the environment (Swaigood, Ellis, Forthman, Shepherdson, 2003). The current study investigated spatial memory in the giant panda, specifically the giant panda's ability to recall information about spatial location.

1.1 Spatial Memory

In general, spatial memory is defined as the ability to remember a location (Clark & Martin, 2005). This capacity has been investigated with a variety of experimental and naturalistic tasks. Spatial memory has been documented across a variety of animal classes; including mammals, birds, and insects (Benhamou & Pouchet, 1996).

The natural history of giant pandas suggests the potential for spatial memory ability. Foraging and/or reproductive efforts may have benefited from the evolution of spatial memory. The giant panda is a carnivore by nature, but has adapted to an almost entirely herbivorous diet which consists primarily of bamboo (Schaller, 1985). The digestive tract of the giant panda is poorly designed for this type of diet, given that it does not have a chambered stomach or the enzymes necessary to break down the cell walls of bamboo. As a result, the digestion of bamboo yields poor nutritional value, forcing the panda to eat large amounts quickly, since very little energy is actually obtained from the consumed food. Schaller (1985) reported that the average distance between bamboo feeding sites ranged from 6.6 ± 5.9 m (species: *Sinarundinaria*) to 9.0 ± 6.1 m (species: *Fargesia*). This short foraging distance suggests that giant pandas may use olfactory or visual cues to locate food sources, which would argue against a need for spatial memory. However, giant pandas appear to be highly selective in terms of bamboo foraging. Giant pandas show preferences for topography of feeding sites as well as distinct seasonal variation in the types of bamboo consumed, parts of bamboo consumed (leaves or stem), and age of plants consumed (Reid & Jinchu, 1991; Schaller, 1985). The seasonal variation in nutrient content of different types of bamboo may guide this complex and fluctuating set of preferences. Spatial memory ability would support remembering the

location of preferred patches throughout the year. Tarou (2003) found that giant pandas use spatial information to locate food.

Another potential driving force for the evolution of spatial memory in giant pandas is the need to locate mates during breeding season. Giant pandas are solitary throughout the year, but will mate with multiple partners during breeding season (Schaller, 1985). Males have larger, less defined ranges and must find females during the breeding season. The ability to remember information about females' home range areas would help males locate potential mates during the breeding season. Much of the interaction between giant pandas occurs through chemical communication. Giant pandas will scent mark in communal locations to convey information and avoid direct contact (White, Swaisgood, Zhang, 2002). Remembering the location of these common scent marking areas is particularly relevant during breeding season. Also, female giant pandas need to remember the location of dens following birth. Spatial memory ability may have evolved because it improves reproductive success in the giant panda.

1.2 Memory in non-human species

The assessment of cognitive factors in non-human subjects is a challenging endeavor; nonetheless it has been attempted and refined in various ways. The focus of the current study was on working or short-term memory. These are commonly studied as separate constructs in the human literature, even though the use of a simple dichotomy is a debatable issue because the two constructs are highly related (Engle, Tuholski, Laughlin, & Conway, 1999). However, the distinction is not made in studies of non-human memory (Washburn et al., 2003). Rather, for the purposes of non-human animal memory research, the concepts of *working memory* and *reference memory* are analogous

to the concepts of human *short-term memory* and *long-term memory*, respectively. Working memory can be considered the “memory for events on a specific trial” and reference memory should be considered the “memory for unchanging characteristics of the task” (Shettleworth, 1998). The current study focused specifically on working memory.

1.3 Recall versus Recognition memory

A variety of approaches have been developed to test memory ability using recognition and recall tasks. In recognition tasks, a single stimulus is shown during a presentation phase, followed by a test phase in which the same stimulus is presented along with additional stimuli. The recognition task requires the subject to identify (‘recognize’) which stimulus had previously been observed, making memory recognition reliant on external stimuli, as the information is generally re-presented. In a recall task, on the other hand, subjects are shown a stimulus during the presentation phase, and then during the test phase are required to reproduce (‘recall’) the stimulus, with no stimulus cues present. Therefore, memory recall relies more on internal cues, because the stimuli cues are limited at the time of retrieval (Craik & McDowd, 1987). In humans, given the lack of external stimuli in recall tasks, these tasks are often verbally based (i.e. “Tell me what you remember”). This scenario allows for a somewhat straightforward approach to addressing memory recall in humans. This situation is not the case in non-human species. Recognition tasks have been used as the primary approach to assessing memory in non-human species because of the difficulty of implementing pure recall tasks (Washburn et al., 2003; Shettleworth, 1998), however, some attempts have been made to involve recall as well as recognition in tasks designed to assess memory. For example,

ordered recognition requires a subject to recognize which objects were previously presented out of an array consisting of both previously presented stimuli and novel alternatives. Additionally, the subject must recall the order in which the objects were initially presented. Another example is the Concentration task, which requires recognition of whether an object has been seen before, and if so, recalling the location (Washburn et al., 2003). However, the majority of memory assessment tasks in non-human species do not focus specifically on memory recall.

1.4 Radial Arm Maze

A standard technique used in the evaluation of spatial memory is the radial arm maze. Olten and Samuelson (1976) developed this apparatus and methodology to evaluate spatial memory and learning in rats. In the radial arm maze, subjects are placed on a center platform, and can travel down eight radial alleys. At the end of each alley, food reinforcement can be obtained. The experiment found that subjects did not use odor or sequence patterns to dictate their arm visitation; rather, subjects relied on spatial memory to avoid returning to already visited arms. The radial arm design has been expanded to large scale apparatus that are capable of testing a variety of species; such as hedgehogs, rabbits, guinea pigs, and a variety of avian species; such as chickens and crows (Lipp et al. 2001). One important development of the radial arm maze, according to Lipp et al. (2001), is the technique of placing food in only half of the maze arms, which allows for a distinction between errors in spatial memory. Spatial working memory errors occur when a subject re-enters a previously baited arm and spatial reference memory errors occur when the subject enters an arm that is never baited (Lipp et al. 2001). Analogs of the radial arm maze are used to test spatial memory in larger

species. Feeders are placed equidistantly around an open space and an animal must visit sites to obtain the food reward. Studies of a variety of species, including sheep and primates have used this methodology to assess spatial memory. For example, Dumont and Petit (1998) demonstrated that sheep are able to remember the location of feeding sites containing a preferred food (pellets). In their experiment, preferred food sites were baited, and nothing differentiated the preferred sites from the rest of the environment. Subjects were more likely to return to the preferred feeding sites, indicating memory for the spatial location.

In primates, an assessment of spatial memory can be conducted with a simulated foraging task adapted from the radial arm maze. MacDonald and Wilkie (1990) tested spatial memory in monkeys using a seminaturalistic foraging task in which feeders (yellow cups containing food) were placed around the subject's enclosure. The subject participated in a walking maze in which all eight feeder sites were baited, and the subject was allowed to freely roam and retrieve food. The order of site visitation was recorded. In the next phase, half of the feeders were baited with food and subjects were allowed to visit and deplete feeders. The subject was then removed from the enclosure, and following a delay (initially 3-5 minutes, but increasing over the course of the experiment), the subject was allowed to return. During the delay, the same four feeders that had previously been baited were again baited (win-stay condition) or the opposite feeders were baited (win-shift). In the win-stay condition, the subjects consistently visited sites that had been previously baited in the prior visit. In the win-shift condition, subjects consistently visited sites that had not been previously baited in the win-shift condition. These findings suggest that the monkeys were accurate at recalling the

location of the food. There is also an indication that the subjects used a least-distance strategy; specifically, the subjects followed a circular pattern, starting from a preferred site. This behavior occurred even though there was no time constraint on the behavior and no incentive (provided by the experimenter) for minimizing travel time.

Tarou (2003) implemented a similar design to examine associative and spatial learning in foraging behavior with giant pandas and spectacled bears. Three male and four female giant pandas located at Zoo Atlanta, San Diego Zoo, and the Smithsonian National Zoological Park were tested with this design. Eight feeders were mounted around an enclosure and food items were placed inside of the feeders. Initially, all feeders were baited and several measurements were made, including the length of time to visit all feeders, the order of site visitation, number of visits to adjacent feeders, and the ability to avoid depleted feeders (which serves as evidence of working memory). On average, the subjects took significantly less time to visit the eight feeders in the last session than the first. The subjects developed a preference for a direction of travel (either clockwise or counterclockwise depending on the subject) and a preference for starting feeder position. The subjects were more likely than chance to visit adjacent feeders than non-adjacent feeders, thus using an efficient foraging strategy. Also, visits to adjacent feeders increased during the last five sessions as compared to the first five sessions. Additionally, there was a significant decrease in the number of revisits to depleted feeders over the course of this task. While the avoidance of depleted feeders is often considered evidence of working memory, the cause is ambiguous in this experiment because the giant pandas also developed an adjacency strategy for visiting feeders. This pattern of responding may be the explanation for a decrease in revisits to depleted

feeders, rather than working memory. Additional research is necessary to clarify whether the pandas use spatial working memory.

Subjects were also tested with a spatial and a reversal task to test the ability of the subject to use spatial cues to determine food locations. In the spatial task (analogous to the win-stay task), four feeders were baited (feeders 1, 4, 5, 7) for each trial and correct visits were those made to baited feeders and incorrect visits were those made to unbaited feeders. Five of the subjects were able to use spatial cues to locate food within 30 trials (criterion required subjects to make 3 correct choices in the first 4 visits on four out of five sessions). To determine whether cues other than spatial cues were being used to locate the feeders, a reversal task was included.

In the reversal task (analogous to the win-shift task), the four originally baited sites were unbaited in the test phase, and the originally unbaited sites were baited. This task was included to determine whether the subjects were relying on visual and olfactory cues to determine food location, rather than spatial cues. If the subjects returned to the originally baited sites (feeders 1, 4, 5, 7), rather than the presently baited sites (feeders 2, 3, 6, 8), this would serve as evidence that the subjects were relying on spatial memory rather than visual or olfactory stimuli to locate the food. On the other hand, if subjects initially visited the presently baited sites (feeders 2, 3, 6, 8), this would serve as evidence that spatial memory was not the only cue being used to locate food and that olfactory or visual cues may be used as well. Initially in this task, subjects that had learned the spatial task visited the originally baited sites (even though they were not baited). This disturbance in performance suggests that the subjects were not using olfaction or vision

to locate the baited feeders. The findings of this study suggest that giant pandas are able to use spatial reference memory to locate food sources.

However, there are some potential confounds to these findings. The subjects adopted a systemic pattern of travel, specifically visiting adjacent locations and starting from a particular location. While this results in efficient foraging, performance greater than chance may result from this strategy rather than from the ability to remember spatial cues. The data also suggest that olfactory and visual cues were not the primary guide, as evidenced by the subject's failure in the beginning of the reversal task, however these alternative factors cannot be sufficiently ruled out by the design of this task.

The radial arm maze paradigm has been an extremely useful design in the evaluation of spatial memory in a multitude of species (Lipp et al., 2001). Additionally, it has provided support for the existence of spatial memory in giant pandas. However, additional tasks and approaches have been used to study spatial memory.

1.5 Delayed Response Tasks

Another popular approach to assessing memory is the delayed response task. In a delayed response task, information necessary to complete the task is given prior to the onset of a delay, after which a response will be made based on the pre-delay information (Tavares & Tomaz, 2002). The delay prior to the test phase is one of the most important factors affecting performance, because the delay increases memory demands (Clark & Martin, 2005). There have been many variations of this approach used to test memory in non-human species.

Hunter (1913) conducted early non-human research using a delayed response design. His design, using contemporary terms, would be labeled as a test of memory

recall, although he does not use this terminology and refers to the experiment as an investigation of “mammalian behavior under conditions where the determining stimulus is absent at the moment of response” (Hunter, 1913, p.1). The experiment required a delayed response to a stimulus (light) for dogs, rats, raccoons, and children. An appropriately sized apparatus was constructed for each species. The subject was restrained in front of three doors, and a light above one of the doors would briefly be turned on, and then turned off. Following a delay, the behavior of approaching the door where the light had been on was reinforced with food. The delays between the light going off and the subject being released to approach the door were increased until subjects failed. At this point, delays were decreased incrementally until performance rates returned to a high percentage correct (varying from 70% to 95% for different subjects). One potential cue that may have been related to successful performance, in addition to recalling the location, was the use of olfactory cues from the food odor to determine correct response (Hunter, 1913). The experimental outcome does not support use of this extraneous cue because some of the responses were incorrect, which should not have occurred if subjects were cued by olfaction. Interestingly, some of the subjects also performed well by orienting their bodies or heads toward the correct door during the delay. The ability of a subject to orient itself as a cue during the delay period must be addressed and prevented by any experiment attempting to evaluate spatial working memory (Hunter, 1913; Shettleworth, 1998).

Medin (1969) tested rhesus monkeys' response to a light pattern using a 4X4 matrix. One, two, or four cells were lighted for one second, and then extinguished. Following a delay of 0s, 1s, 2s, 5s, 10s, or 20s, the subject was required to open the door

to the previously lighted square or squares. The subject was allowed to make one response per number of squares lighted in a trial, and a correct response was reinforced with a food reward and the light coming on. For the condition in which only one cell was lighted, performance (percentage of successes) significantly decreased due to a main effect of the delay. An interesting finding of this study was an interaction between age and delay, with the older subjects performing significantly worse on the longer delay trials, as compared to the shorter delay trials. The intent of this study was to investigate reproduction of patterns, but the methodology is useful in designing an apparatus to address memory recall.

Wilkie and Summers (1982) describe Medin's approach as a useful method for studying working memory for spatial cues in animals. Wilkie and Summers developed a task in which pigeons were required to respond to a 3X3 matrix of squares. One square would be lighted, and following a delay, the previously lighted square and an additional one (or more) squares were lighted. The subject had to respond to the original square by pecking it. This is a delayed match to sample response, because the original stimulus (sample) is present at test time, and the subject must recognize and respond to the sample to correctly perform the task. Thus, this task represents a recognition task, rather than a recall task. A simple methodological adjustment would transform this task into a test of memory recall. Specifically, if the lights were not re-presented at test time, this design would function as a test of recall. This study effectively addressed spatial working memory because other than the location, the squares were identical. An additional finding was that performance decreased as a function of increasing delay. Performance was above chance for delays of 1s, 2s, or 4s, but below chance for 8s and 16s delays.

Wilkie and Summers (1982) urged the use of this technique, in addition to paradigms such as the radial arm maze, to investigate spatial working memory.

In 2003, Washburn et al. attempted to understand why rhesus monkeys (*Macaca Mulatta*) performed at or below chance on a visuo-spatial memory task. Specifically, they tested whether the deficit occurred in visual memory, spatial memory, or in the integration of the two. For the spatial memory component, grids were presented on a computer screen, with variations from 2 to 12 cells. The cells contained dots that would be rewarded with a beep and food when touched with a cursor. However, after a dot had been rewarded once, a return visit to the already visited dot would result in a buzzing sound and no reinforcement. Thus, if a subject remembered the locations previously visited, it would not return to the location. This ability was taken as evidence for spatial memory because other than location, the dots were identical in appearance. The results were that the subjects performed better than chance, although worse than the human subjects. Another interesting finding was that the human subjects adopted a simplifying strategy for “collecting” dots (clockwise pattern), but the monkey subjects did not develop a strategy. Because the monkeys failed to develop a strategy, researchers were able to assert that spatial memory was being used to perform the task.

Washburn et al. (2003) developed an additional task designed to test spatial memory specifically. In this task, a circle was surrounded by two to five squares on the computer screen. A target stimulus would appear in one of the squares, then disappear, and after a retention interval, reappear in the circle in the center of the screen. To successfully complete the task, the subject was required to move the target stimulus back to the square in which it had originally appeared using a joystick. The subjects

performed above chance in this task, indicating that the subject recalled where the stimulus had been located. This task is a recall task because the subject had to recall the previous location of the stimulus in order to return it to the original square.

Washburn et al. (2003) conducted spatial memory recall tasks in rhesus monkeys using a computer and joystick. This methodology presents a useful technique for testing this ability; however, this approach is not conducive to testing with all species.

Specifically, the computer and joystick approach may be a challenge to implement in research involving species that are large, cannot be trained to use a joystick, or that lack visual acuity.

The underlying assumption of delayed response tasks is that they assess memory, which erodes over time. According to Clark and Martin (2005), because an increase in the delay places more demand on memory, impairment in performance across delays can be interpreted as evidence that a task is addressing memory. This assumption is made because the delay-dependent impairment excludes other explanations, such as perception, motivation, or attention deficits. However one must be careful about the conclusions made from delayed response studies.

While memory and time are undoubtedly related, it should not be assumed that memory is a simple function of time. The method in which a subject is trained to respond to a delayed response task may have a significant impact on performance. Some research suggests that delay induced impairment in performance (i.e. the forgetting function) may be the result of a generalization gradient along a temporal dimension (White, 2001). In a match-to-sample task, remembering is a discriminative behavior and delay time to response is considered another dimension of the stimulus, similar to any other physical

dimension of the stimulus (e.g. wavelength). The forgetting function is to some extent a result of the task training occurring at a 0 second delay and the subsequent testing delays being of greater length (Sargisson & White, 2001). Sargisson and White (2001) demonstrated that pigeons trained to respond after different delays from the start of training (0s, 2s, 4s, 6s) showed generalization gradients around the initially trained delay time when a variety of test delay times (0s, 2s, 4s, 6s, 10s) were used. The greatest accuracy occurred at the initially trained delay time, even when the test delay times were shorter than the trained delay time. For example, subjects trained at a 6 second delay demonstrated maximum accuracy at the 6-second delay, and accuracy declined for both shorter and longer delays. This contradicts the traditional notion that forgetting is a simple function of time and that the best performance should occur with no delay, and that performance will decrease as the delay increases. The results of Sargisson and White (2001) support that the delay at which subjects are trained at least partially contributes to the decline in accuracy and performance as the delay is increased in memory tasks in pigeons. Furthermore, an increase in the delay to reinforcement delivery will result in a decrease in response rate, also known as a delay-of-reinforcement gradient (Reilly & Lattal, 2004). In delayed response tasks, even if reinforcement is delivered immediately following a correct response, the reinforcement is delayed in relation to other aspects of the task (e.g. the behaviors of attending to and remembering the correct information). Ferster (1953) found that by gradually increasing the delay, normal response rates can be maintained across delay.

The current study increased delay durations between the presentation of the stimulus and the response in a progressive and gradual manner. Subjects had to reach

criterion on each delay before beginning training on a longer delay. Subjects were given several sessions to train for each successively longer delay. Performance was still expected to decline with longer delays, but the incremental increase in delay times was expected to prevent a sharp drop-off in performance as seen in other delayed response tasks. This method was used to minimize the effect of dissimilarity in testing delays from training delays, and more accurately assess memory ability. Reinforcement delivery immediately followed a correct response on all trials.

As previously discussed, Tarou (2003) found that giant pandas have spatial memory ability, yet there was a need for additional work to further explore this cognitive ability. The current study completely eliminated the possibility of olfactory cues being used as the main source for location because a light was used to indicate a “baited” site, rather than actually baiting the site with food reinforcement. This design of the current apparatus also prevented the subject following a rule of thumb, such as walking in a clockwise circle, visiting adjacent locations, or adapting another simplifying strategy. The methodology and apparatus were adapted from previous approaches to researching spatial memory, and specifically focused on the giant pandas’ ability to recall spatial location information.

The study used an apparatus constructed with a 2x3 grid facing the subject, with a light mounted behind each square. After training was completed, the task required the subject to remain stationary during the presentation of a light behind one of the squares, and continue to remain stationary for a delay period of 2-seconds during phase 1 of testing. A target stick was used to restrict the possibility of the subject orienting its body as a cue during the delay. After the delay, the subject was given a cue to approach the

apparatus and select the square where the light was previously located. If a subject reached criterion for this phase, various delay times were used (3s, 4s, 5s, 6s, 10s, 15s, 20s) before the subject was cued to respond.

It was hypothesized that subjects would successfully perform the task of selecting the previously lighted location with the 2-second delay condition (8 out of 15 correct, binomial distribution, $p < .05$). It was also expected that a subject's performance (% correct responses) would decrease as the delay increased. Because the delay intervals were increased incrementally, a sharp drop-off in performance was not expected, rather a gradual decline. Successful training of the task and use of the apparatus served an important function in the study of spatial memory recall.

CHAPTER 2

METHOD

2.1 Subjects

Subjects were one male and one female adult giant panda on loan to Zoo Atlanta from the Chengdu Research Base of Giant Panda Breeding. The male (Studbook #461) was born 9 September 1997. The female (Studbook #452) was born 25 August 1997 (Xie and Gipps, 2003). They are housed on public exhibit from approximately 0900 – 1700 (indoor and/or outdoor exhibit) and in an indoor holding enclosure during other hours. Research was conducted in the indoor holding enclosure before the animals go on exhibit (0800 – 0830). The male subject was trained and tested from August 2006 – April 2007 and the female was trained and tested from April 2007 – June 2008. Training or test sessions occurred approximately three times per week.

2.2 Apparatus

A wooden structure was divided into a grid pattern, forming six squares. Each square was covered with Plexiglas, and a light source (7 watt bulb) was attached behind each square of Plexiglas. The apparatus could be attached to the mesh of the enclosure on the experimenter's side. The Plexiglas grid faced the subject. A piece of PVC pipe extended from the center of each square approximately 30 mm into the enclosure and each extension was capped. Henceforth, these capped PVC pipe extensions will be described as "buttons". A target stick made of PVC pipe emerged from a hole in the center of the apparatus.

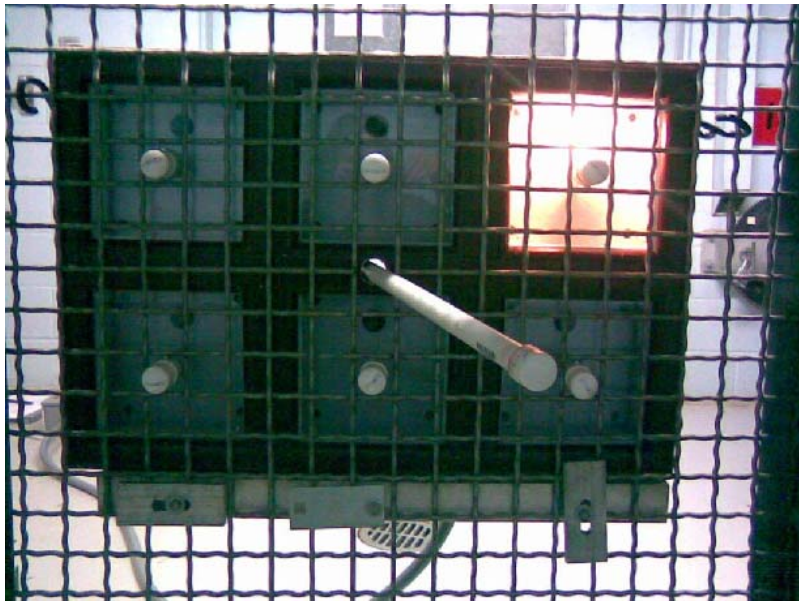


Figure 1. Subject view of apparatus. Light indicates baited site.



Figure 2. View of target stick and buttons.

The target stick was capped, could be inserted approximately 46 cm into the subject's enclosure, and could be shifted left or right.

The dimensions of the apparatus were 50.8 cm x 76.2 cm x 15.2 cm (height x width x depth). Each square was 18 cm by 18 cm and served as a "bait site." Each light source could be operated and controlled independently of the other lights with a dial that altered the brightness of the light from full brightness to no light. Full, medium, and no light brightness levels were specified on the dial. Subjects were trained to push "buttons" which were connected to a spring. When pushed by the subject, the spring contracted and completed a circuit. On the experimenter side of the apparatus, the completion of this circuit resulted in a small light turning on (only visible on the experimenter side).

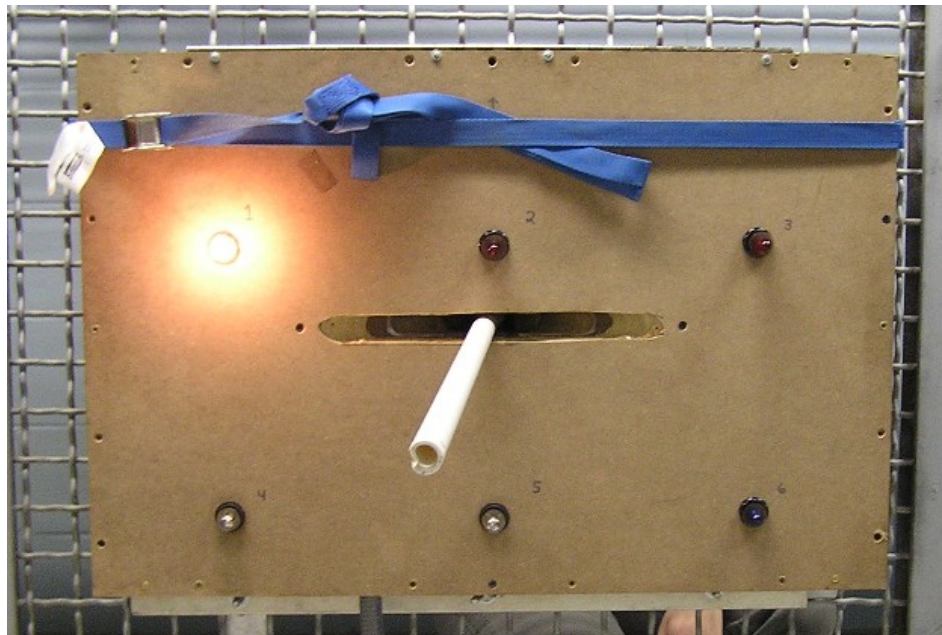


Figure 3. Experimenter view of apparatus. Lighted site indicates subject response.

Thus, the responses of the subject could be monitored with no interaction between the experimenter and subject, which prevented cueing. The apparatus was also large enough that it completely occluded the subjects' view of the experimenter and vice versa.

2.3.1 Training Procedure

Subjects were trained to use the apparatus using positive reinforcement. This method has been successfully used in previous training with giant pandas, and in particular with these subjects (Bloomsmitth et al., 2003; Kelling et al., 2006). Correct responses were reinforced with an auditory reinforcer (i.e. whistle) immediately followed with food reinforcement. Food reinforcement consisted of five food items: banana, apple, pear, sweet potatoes, and leaf-eater biscuits. These items are regularly provided to the animals for training reinforcement and are part of the subjects' daily diet. The amount used as reinforcement for the experiment was similar to the quantity provided in a standard feeding session, which is approximately 100g. Each giant panda was provided with the regular amount of bamboo each day for the duration of the experiment. Banana, apple, pear and sweet potato were cut into approximately 2.5 cm x 5 cm pieces. Leaf-eater biscuits were broken in half, resulting in pieces approximately 2.5 cm x 5 cm. Any food not earned as a reward during this task was given to the subjects later that day. Food reinforcement was delivered to the subject through the mesh of the enclosure.

2.3.1.1 Initial training

Initial training was conducted on a two-square prototype apparatus (36 cm x 48 cm x 15 cm). One light was turned on and the behavior of pushing the button of the lighted square when given the verbal command "light" was reinforced. The lighted site was randomly determined for each session. The two-square apparatus could be mounted

in a vertical or horizontal position. After the subject responded correctly to 10 out of 15 trials in both the vertical and horizontal orientations, this behavior was transferred to the six-square apparatus. Subjects were trained on the six-square apparatus until a criterion of 10 correct out of 15 trials was achieved. After the behavior of pushing a lighted square when given the command “light” was established, the next phase of training began.

2.3.1.2 Brightness Fading

Subjects were trained to remain stationary during observation of the light and during a delay following the presentation of the light. This training was accomplished by using a target stick. The target stick was inserted approximately 30.5 cm into the enclosure.



Figure 4. Subject trained to touch target stick while light is presented

The subject was trained to touch its nose to the end of the target stick and hold that position for three seconds while one of the lights was turned on. The target stick was then shifted to a randomly predetermined side position, either right or left. The behavior of touching the target stick in the side position was reinforced with the auditory reinforcer (i.e. whistle). As the subject targeted in the side position, the saliency of the light was changed from full brightness to medium brightness. The target stick was returned to the center position and the subject was required to target it again. Then the target stick was withdrawn and the command “light” was given. The subject had to approach the apparatus and push the correct square (which was at medium brightness). Correct responses resulted in auditory and food reinforcement. The behavior of repositioning of the head was trained to prevent the subject from maintaining eye contact or postural contact with the light stimulus.

2.3.1.3 Time Fading

After subjects responded to 10 out of 15 trials correctly, the brightness fading technique was combined with a time fading procedure. The same procedure was followed, with the light faded to medium brightness during the side target. After the subject returned to the center and targeted, the target stick was withdrawn and the command “light” was given. As the subject approached the lighted site (medium brightness), the experimenter slowly faded the light until it was extinguished. At first, the light was not fully extinguished until the subject was actually pushing the button. As the training progressed, the light was dimmed more rapidly and was fully extinguished as the animal neared the apparatus. The experimenter continued to dim the light more rapidly until it was fully extinguished when the subject began to approach the apparatus.

Then, the light was fully extinguished before the animal was prompted by the withdrawal of the target stick and the command “light.” Finally, the light was turned off completely as the subject targeted to the side position. After the subject reached criterion for this procedure (8 out of 15 correct), the delayed response phase of testing began. A non-correction procedure was used because the stimuli were no longer present at the time of response. It was assumed that if the subject did not recall the location on the first attempt, further responses would reflect a guessing strategy which should not be reinforced.

2.3.2 Testing Procedure

2.3.2.1 Phase One: Two-second delay

Subjects were required to respond to a previously lighted location following a 2-second delay. There were 15 trials per session and the correct locations were randomly determined for each session. There were two experimenters present for every session. Experimenter 1 stood behind the apparatus and was responsible for moving the target stick, providing auditory reinforcement (i.e. a whistle) for correct targeting behavior, and prompting a response from the subject after a delay. Experimenter 1 also delivered food reinforcement for correct responses. Experimenter 1 was blind to the correct location on every trial. Experimenter 2 sat behind experimenter 1 and the apparatus and controlled the lights via a remote dial. Experimenter 2 was responsible for turning on and off the lights, starting the timer, and providing auditory reinforcement for correct responses.

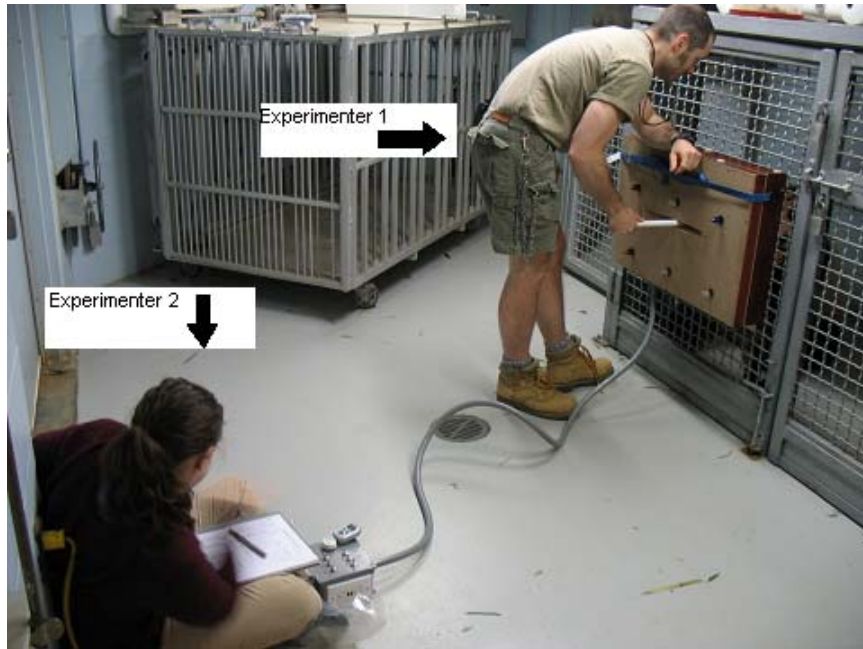


Figure 5. Illustration of the experimenter positions.

A trial began when the subject touched the target stick with his/her nose in the center position. This behavior resulted in auditory reinforcement and the correct location was lighted for three seconds. The subject was then required to follow the target stick to a side position and touch it with its nose. This behavior was reinforced with the auditory stimulus (i.e. whistle). At this point, the light was extinguished and the delay began. The target stick was then returned to the center and the subject was required to touch its nose to the target stick in the center position. The subject remained in this position until an auditory stimulus (vibrating timer) cued experimenter 1 that the delay had elapsed. At this point, the target stick was withdrawn and the subject was prompted by the command “light” to approach the apparatus and push the previously lighted square. A correct response resulted in auditory reinforcement followed by the delivery of food. An incorrect response resulted in the auditory stimulus “NO,” followed by an approximately

five-second intertrial interval. Subjects remained in this phase of testing until performance reached a criterion value of 8 out of 15 correct responses. This is statistically significantly different than chance at the $p < .05$ level. Because there were six response options, the random chance of responding correctly on a trial is only 16.6667%. Thus, in a session with 15 trials, subjects would only be expected to respond correctly approximately 2.5 times per session if responses were random. As modeled by a binomial distribution, 8 out of 15 correct responses is significantly different than chance (≈ 2.5 out of 15).

2.3.2.2 Phase Two

The intention of this phase of testing was to explore how performance changed with increasing delay. To achieve this, the same method described above was used, and longer delays were added. However, an abrupt shift to longer delays was expected to alter and disturb performance, perhaps to the extent that subjects would no longer participate. Therefore, increased delay times were gradually included. This incremental increase in delay also allowed for training to take place at each delay time. Furthermore, to monitor for confounding influences, the 2-second delay was included in all sessions. If the performance on the 2-second delay trials in a session fell below chance, it could not be expected that the performance on the longer delays would accurately reflect the subjects' ability to respond following a longer delay. Rather, it may reflect a subject's lack of motivation, a high level of distraction, or perseverative responding during that session. Essentially, the 2-second delay served as the yardstick or baseline for performance. If performance at the 2-second delay remained stable, the responses to the longer delays were considered an accurate reflection of ability.

Sessions proceeded as follows. After reaching criterion performance for the 2-second delay, the sessions included 3-second delays. The sessions included eight 3-second trials and seven 2-second trials. Criterion was 4 out of 8 correct responses for the longer delay ($p < .05$). Performance on the 2-second delay was required to be 4 out of 7 correct responses ($p < .05$). If performance fell below criterion for the 2-second delay on all sessions during phase two, the experimenter evaluated and attempted to resolve any confounding factors. Subjects were tested for 32 trials (4 sessions) to determine if they could be trained to respond accurately at a 3-second delay. Testing was considered complete for the 3-second delay at any point in which criterion was met.

For any subject that reached criterion, a 4-second delay was added. There were eight 4-second delays and seven 2-second delays. The procedure proceeded as described above. For any subjects that reached criterion for a 4-second delay, the same procedure was repeated for the 5-second delay, and so forth. The delay times used were 3-, 4-, 5-, 6-, 10-, 15-, and 20 seconds.

Testing was considered complete for a subject when it failed to respond correctly to the longer delay for four sessions while maintaining accurate performance on the 2-second delay.

2.4 Data Analysis

To ensure that acquisition of the task was not because of chance, the calculation of reaching criterion based on random chance was made. A criterion was selected that limited the probability of reaching criterion by chance to less than 5%.

For the initial phases, in a given set of trials, the criterion was 8 out of 15 ($p < .05$). Using a binomial distribution, the probability of a subject correctly selecting the

lighted bait square by random chance on 8 or more trials out of 15 was less than 5%. For the delayed response phase of testing, subjects had to reach a criterion of 4 out of 8 correct responses on the longer delay trials ($p < .05$). Using a binomial distribution, the probability of the subject correctly selecting the bait square by random chance on 4 or more out of 8 trials is less than 5%. Subjects were also required to maintain an accurate level of performance on the 2-second baseline delay trial (4 out of 7, $p < .05$)

CHAPTER 3

RESULTS

Given the small sample size, the results are limited to the spatial memory recall ability of the subjects in the study, and no inferences regarding the population will be made.

The male subject, Yang Yang, reached criterion for phase one of testing (8 out of 15 correct responses in one session). In phase two of testing, he reached criterion for 3-, 4-, 5-, 6-, and 10-second delays (4 out of 8 correct responses in one session).

The female subject, Lun Lun, reached criterion for phase one of testing (8 out of 15 correct responses in one session). In phase two of testing, she reached criterion for 3-, 4-, 5-, 6-, 10-, and 15-second delays (4 out of 8 correct responses in one session).

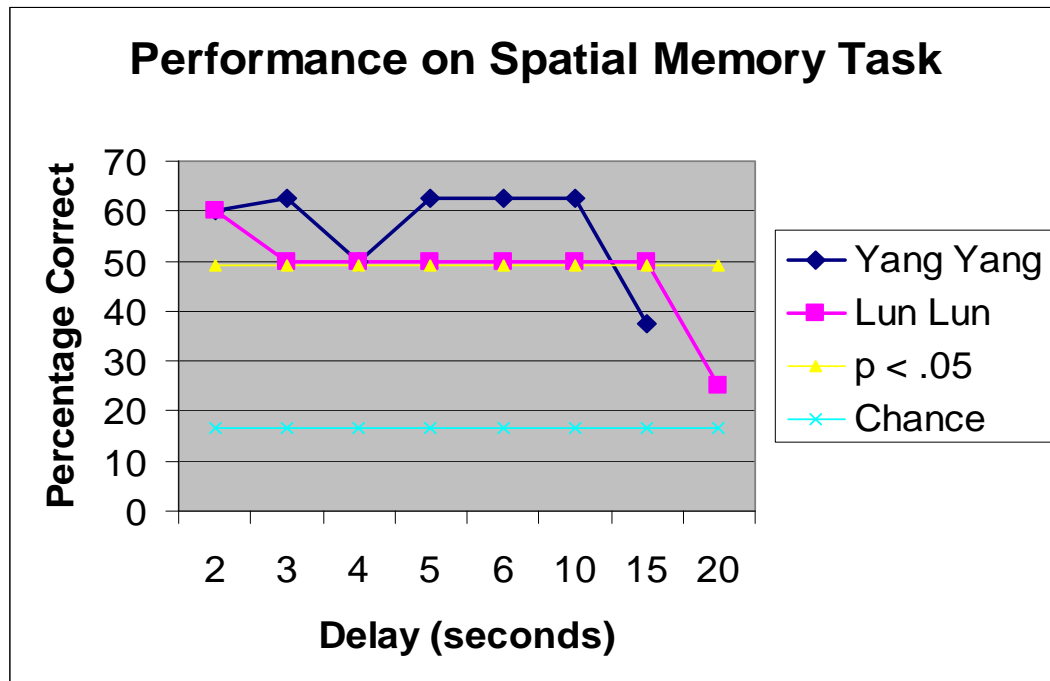


Figure 6. Percentage correct on last session for each delay

CHAPTER 4

DISCUSSION

The results support the hypothesis that giant pandas are able to recall spatial information after a short delay in the absence of external cues. The current design eliminated the possibility that subjects could use olfactory or visual cues to remember location information because the locations were indirectly baited with light, rather than food. The results also support the hypothesis that memory performance declines as the delay increases, suggesting that this method successfully tests memory ability. A sharp decline in performance with increasing delay did not occur, which can most likely be attributed to the gradual and incremental increase in delay times used for testing. The results suggest that giant pandas reach a threshold after an approximately 10-15 second delay in which recalling spatial information becomes increasingly difficult.

It is possible that subjects could have performed above chance for longer delays if more training sessions were allowed. However, this number of sessions was sufficient to reach criterion at all shorter delays. Additional training and research may reveal slightly different ability levels. Also, it may be easier to remember a location where food is actually present, rather than remember a location where a response will result in food, but is not actually retrieved from that location (Wilkie & Summers, 1982; Wilkie & Willson, 1993). Nonetheless, the results provide evidence that giant pandas can recall location after short delays without external cues.

A potential problem in spatial memory recall tasks is the ability of an animal to use its own body as a cue during the delay (Shettleworth, 1998). This study was designed to prevent eye and/or postural cueing. Subjects had to turn their heads and bodies away

from the apparatus as the light was being extinguished, preventing potential cueing. The design of the apparatus also served to prevent cueing from the experimenters. The experimenter prompting the subject to move with the target stick was blind to the baited location on each trial. The experimenter controlling the lights and reinforcing correct responses could not see the subject, nor could the subject see this person. The apparatus completely occluded the view between the subject and this experimenter. Although cueing is a potential problem in animal research, all efforts were made to prevent it in this study. The development of a more automated design might further reduce the possibility of cueing.

Factors such as motivation, distraction and perseveration may affect performance on delayed response memory tasks. Sessions were conducted in the morning as an attempt to ensure food motivation and to minimize distraction. Two-second delays were included in all sessions with longer delay times. The subject would not be considered to fail a longer delay if performance on the two-second delay fell below chance for all testing sessions. This would suggest that the subject's responses were no longer an accurate measure of memory ability if the subject failed to pass the shortest delay, perhaps as a result of perseverative or unmotivated responding. This did not happen for either subject and performance on the shortest delay remained above chance when the performance on longer delays fell below chance. This study used techniques to ensure that memory ability was tested across delay, but the potential influence of confounding factors needs to be considered and monitored for in any study of this design.

Sex differences in spatial memory were not explicitly tested because of the small sample size, but several interesting patterns emerged. Although any variation in

performance may be because of many possible individual differences between the two subjects, there is a possibility that the differences relate to the substantial field of research indicating that males outperform females in spatial tasks (Jones, Braithwaite, & Healy, 2003). This difference is one of the most studied and supported sex differences in cognition; specifically, that in many mammal species, males outperform females on a variety of spatial tests (Jones et al. 2003). Many evolutionary theories have been developed to explain this difference, but a review of the current theories determined that the range size hypothesis has far more empirical support than any of the alternative theories (Jones et al., 2003). The range size hypothesis, developed by Gaulin and Fitzgerald (1986), predicts that sex differences in spatial ability will be found in a species that has a mating system that selects for different range sizes depending on sex (promiscuous, polygamous, or polyandrous). Both male and female giant pandas have multiple partners, thus a promiscuous mating system, and there is evidence indicating that male giant pandas inhabit larger home ranges than female giant pandas. According to Schaller (1985), females tend to inhabit an area with a well-defined core, and the male roams a wider area, with no defined core. Liu, Skidmore, Wang, Yong, and Prins (2002) used radio tracking to also conclude that male giant pandas, on average, travel larger distances than females. Based on their range sizes and mating system, it is expected that giant pandas will show sex differences in spatial ability.

The results of the current study provide some preliminary support for the range size hypothesis. The female took 91 sessions to train for the task, while the male took only 65 sessions to complete training. The male also had a higher percentage correct passing score for the 3-, 5-, 6-, 10-second delay times than the female, and equivalent

scores for 2 and 4 second delays. These findings suggest that giant panda males may have better spatial ability than females. However, the female reached criterion for a fifteen second delay, and the male only reached criterion for a ten-second delay. This particular inconsistency with the range size hypothesis is not entirely surprising given the nature of the current task, which was designed to test working (short-term) recall ability. Sex differences are typically found in reference (long-term) memory ability, which would be impacted more significantly by the evolutionary pressures previously described. A methodology designed to test reference spatial memory should be used with a larger population of giant pandas to test the range size hypothesis.

Another interesting application of this methodology would be to evaluate age differences in performance. Medin (1969) cites memory loss, rather than decreased attention or motivation, as a probable cause of inferior performance of older rhesus monkeys as compared to middle-aged monkeys over longer delays on a pattern reproduction task. Additionally, spatial memory appears to be impaired in older rats as compared to younger rats (Rapp, Rosenburg, & Gallagher, 1987; Shukitt-Hale, McEwen, Szprengiel, & Joseph, 2004). Many studies of humans have also shown a decrement in memory performance with age; specifically, more substantial decrements on recall tasks as compared to recognition tasks (Craik & McDowd, 1987). This methodology could be used to test for age related differences in spatial recall memory performance in giant pandas, as well as other species.

An important outcome of the current study is the development of a new task capable of testing recall ability in previously untested species. As previously discussed, the majority of memory studies in non-human species, including spatial memory, have

been conducted with recognition tasks (Washburn, Gullledge, & Martin, 2003). Hence, memory recall in non-human animals is a largely neglected area of research. This apparatus could be used to address this issue in many species which are not suitable candidates for computerized testing.

This research significantly adds to our understanding of giant panda spatial memory. Previous research found that giant pandas use spatial cues to remember the location of food (Tarou, 2003). The results of the current study confirm this outcome and broaden our understanding of spatial memory ability. Giant pandas are able to recall location information without external stimuli to guide their behavior. A larger sample of giant pandas should be tested to progress the currently limited knowledge of cognition in this species. Furthermore, this procedure and apparatus should be used to test additional species and expand our knowledge and understanding of spatial memory recall in non-human species.

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